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Gelatin, proteins, Alcoholic Beverages
Starch, Emulsions.
Dairy products, Sugar
Technology. ~~Fruits~~ Jellies, Jams,
marmalades, ~~Brewing~~,

COLLOID ASPECTS OF FOOD CHEMISTRY AND TECHNOLOGY

BY

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Colloid aspects

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PREFACE

WHILST there are several textbooks available in English, German and French which deal with the organic chemistry of foods, the chemical aspects of nutrition and with food analysis, no attempt seems to have been made to survey the field in relation to those physico-chemical aspects of food chemistry which come within the range of modern colloid chemistry. The present volume seeks to fill this gap in the textbook literature.

It is not a treatise on food technology, but rather an introductory guide to those aspects and problems of a colloid nature which may be unfamiliar to the food chemist who has not specialised in colloid chemistry and physics.

The literature is voluminous and scattered throughout a great number of journals and research bulletins. In attempting to summarise the main features of the subject, very full references to original papers have been given, enabling the student to follow up any desired point in detail. The patent literature has not been ignored.

I have assumed an elementary knowledge of colloid chemistry, and apart from a very brief introduction dealing with the scope of the present-day position of the science, colloid concepts are developed not according to a regular discipline, but as and where main points of interest arise. At the same time, the order of the chapters follows the usual study of colloid systems : proteins, carbohydrates, emulsions, adsorption, foams. A glossary of terms has been included for the benefit of students unfamiliar with the subject.

Those whose reading in colloid chemistry has passed the elementary stage will readily appreciate that food chemistry and technology provide a field which engages all the important problems of the science. The propositions and queries treated in any of the larger standard works on colloid chemistry find immediate and interesting exemplification somewhere in the range of products coming within the purview of the food chemist engaged in technical operations.

In the hope that the research worker may be assisted, a comprehensive bibliography of over 2,000 references has been compiled, arranged under convenient subject headings.

It is my pleasant duty to express my obligation and thanks to many scientists who have so cordially responded to appeals for

reprints of papers and for permission to use data or to reproduce diagrams or photographs.

Reprints of important papers (usually with permission to reproduce where desired) have been sent me by : Dr. W. D. Bigelow (National Canners Association, Research Laboratories, Washington, D.C.) ; Mr. D. M. Gray (Chairman, Mayonnaise Products Manufacturers Association of America) ; Dr. L. J. Harris (Nutritional Laboratory—Medical Research Council—Cambridge) ; Mr. C. A. Holmquist (Chief Engineer, State Department of Health, Albany, N.Y.) ; Mr. N. King, M.Sc. (State Dairy Station, Tallinn, Reval, Estland) ; Prof. W. C. Mc.C. Lewis, F.R.S. (University of Liverpool) ; the late Prof. J. Loeb ; Dr. Dorothy Jordan-Lloyd (Lister Institute, London) ; Prof. Dr. W. Mohr (Kiel) ; Prof. Ch. Porcher (Lyon) ; Mr. F. H. Waring (Chief Engineer, State of Ohio, Department of Health, Columbia, U.S.A.).

For special permission to reproduce data or illustrations I am indebted to : Prof. C. H. Bailey (Editor-in-Chief, *Cereal Chemistry*) ; Prof. W. D. Bancroft (Editor, *The Journal of Physical Chemistry*) ; Miss Dorothy A. Beckett (Dublin) ; Messrs. The Cherry-Burrell Corporation (Chicago) and their London Director, Mr. F. Rodgers, who obtained permission from Prof. C. D. Dahle of the Pennsylvania State College for the reproduction of photomicrographs of ice creams ; Prof. W. M. Clark (The Johns Hopkins Medical School) ; Mr. A. C. Dahlberg, M.S. (New York State Agricultural Experiment Station) ; Mr. E. G. Eberle (Editor, *The Journal of the American Pharmaceutical Association*) ; Mr. F. D. Farrow, M.Sc. (British Cotton Industry Research Association) ; Dr. R. L. Hill (Utah State Agricultural College) ; Mr. H. E. Howe (Editor, *Industrial and Engineering Chemistry*) ; Dr. O. F. Hunziker (Blue Valley Creamery Company, Chicago, Ill.) ; Dr. K. F. Kellerman (Acting Chief of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D.C.) ; Prof. H. R. Kruyt (University of Utrecht, Holland) ; Prof. A. B. Lamb (Editor, *The Journal of the American Chemical Society*) ; Dr. C. A. McCue (Dean and Director, University of Delaware Agricultural Experiment Station) and his assistants, Dr. F. B. Myers and Mr. G. L. Baker ; Dr. T. Moran (Low Temperature Research Station, Cambridge) ; Prof. Dr. Wo Ostwald (Editor, *Kolloid Zeitschrift* and *Kolloidchemische Beihefte*) ; Prof. Dr. Otto Rahn (Cornell University) and his publisher, Paul Parey (Berlin) ; Prof. W. H. E. Reid (University of Missouri, Columbia) ; The Council of the Royal Society and Mr. F. A. Towle, Assistant Secretary ; The Secretary of the Institute of Brewing and the Editor of *The Journal of the Institute of Brewing ;* Prof. A. W. Thomas (Columbia University) ; Drs. H. I. Waterman and van Aken (Delft-Holland).

For very special favours I am glad of this opportunity of thanking

Dr. McCue and Prof. Dr. Otto Rahn. To the Chemical Catalog Company Inc. of New York I tender my best thanks for permission to quote from Alexander's *Colloid Chemistry* and Walton's *A Comprehensive Survey of Starch Chemistry*.

The task of preparing my book has been lightened by: Mr. W. F. Clifford (Librarian of the Chemical Society, London) always helpful and courteous; Mr. E. G. Turner, who prepared all the diagrams in a form suitable for reproduction; Miss Sylvia Thimidis, who expeditiously and accurately rendered the typescript, and finally Messrs. J. and A. Churchill, whose patience and consideration were once again made evident to me.

WILLIAM CLAYTON.

ORPINGTON.

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COLLOID ASPECTS OF FOOD CHEMISTRY AND TECHNOLOGY

CHAPTER I

THE SCOPE OF COLLOID CHEMISTRY

The first detailed study of colloid chemistry began with Thomas Graham, although certain earlier works certainly developed ideas anticipating in some measure the conclusions of Graham. Thus, Baudrimont [1] and Selmi [2] deserve to be more widely known for their work, which has much interest in the history of colloid chemistry.[3] Again, Acherson [4] (1840) anticipated certain comparatively modern views bearing on emulsions.

THE WORK OF GRAHAM

Graham worked primarily on the problem of diffusion in solution,[5] introducing the technique of dialysis. To him we owe important terminology [6] in colloid chemistry. Graham observed that when certain solutions were separated from the pure solvent by organic membranes, *e.g.*, parchment, the solute could either pass through the membrane rapidly or it either failed to pass or did so very slowly. Accordingly, two main classes of solutions were apparent on the basis of capacity for diffusion through membranes, leading Graham to speak of " Liquid Diffusion Applied to Analysis." Such dialysis tests revealed diffusible and non-diffusible substances, the former being, as a rule, crystalline and the latter amorphous, such as glue, starch or gums. The second class Graham named colloids.[7] In his own words :

" Low diffusibility is not the only property which the bodies last enumerated possess in common. They are distinguished by the gelatinous nature of their hydrates. Although often largely soluble in water, they are held in solution by a most feeble force. They appear singularly inert in the capacity of acids and bases and in all the ordinary chemical reactions. But, on the other hand,

[1] *Vide* " Les Origines de la Chimie Colloidale," A. Baudrimont (1806—1880), par Paul Bary (Paris, 1929).

[2] *Vide* Hatschek, " Foundations of Colloid Chemistry " (London, 1925), pp. 31—62.

[3] A good introduction to the subject is given by Kopaczewski, " L'Etat Colloidal et L'Industrie," Tome I., Chap. I. (Paris, 1925). See also Alexander, *Science*, **61**, 184 (1925).

[4] *Vide* Hatschek, *loc. cit.*, p. 26.

[5] *Phil. Trans.*, **151**, 183—224 (1861) ; *Proc. Roy. Soc.*, **13**, 335 (1863—64).

[6] *J. Chem. Soc.* (1864), 618—26.

[7] $\kappa\omega\lambda\lambda\alpha$ = colle.

their peculiar physical aggregation with the chemical indifference referred to appears to be required in substances that can intervene in the organic processes of life. The plastic elements of the body are found in this class. As gelatin appears to be its type, it is proposed to designate substances of this class as colloids, and to speak of their peculiar form of aggregation as the colloidal condition of matter. Opposed to the colloidal is the crystalline condition. Substances affecting the latter form will be classed as crystalloids. The distinction is, however, no doubt one of intimate molecular constitution."

The fundamental idea in Graham's views was that complexity of molecular structure was the key to matter being in the colloidal condition. He states : " The inquiry suggests itself whether the colloidal molecule may not be constituted by the grouping together of a number of smaller crystalloidal molecules, and whether the basis of colloidality may not really be this composite character of the molecule." That his crystalloid-colloid classification was non-rigorous is not sufficiently well known, yet he clearly stated : " Ice itself presents colloidal characters at or near its melting -point " and " albumen . . . is seen to assume a crystalline contour. Can any facts more strikingly illustrate the maxim that in nature there are no abrupt transitions and that distinctions of class are never absolute ? "

UNIVERSALITY OF THE COLLOID STATE

Just how the transition from colloid to crystalloid state or *vice versâ* could be made to occur was a mystery to Graham and it remained for von Weimarn, in 1905—1906, to commence the working out of his theory of the universality of the colloidal state. He was " thoroughly convinced that ' crystal ' and ' colloid ' are not antithetic concepts, are not two separate ' worlds of matter.' "

von Weimarn [1] has amply demonstrated by numerous experiments his fundamental principle that " all the properties (both chemical and physical) which earlier were considered as specific for colloid substances, appear spontaneously with *every* solid substance as its subdivision progressively increases, irrespective of the means by which this subdivision is brought about (*e.g.*, whether they be chemical, physico-chemical, physical, mechanical and the like)."

Modern colloid chemistry admits that, under suitable conditions, any solid may be obtained in colloidal condition in a suitable liquid medium, the criterion of colloidality being a certain dimension-range of the particles.[2] In fact, *all* substances can appear in the colloidal state under appropriate conditions.

[1] " Die Allgemeinheit des Kolloidzustandes," Bd. I. (Dresden, 1925) ; Alexander's " Colloid Chemistry," Vol. I., 27—101 (New York, 1926).
[2] McBain and Kistler, *J. Phys. Chem.*, **35,** 130 (1931).

CLASSIFICATION OF COLLOIDS

Various attempts have been made to classify colloidal solutions, but the generally accepted classification is a broad one by Wo. Ostwald [1] based on the physical state of the substance present in colloidal conditon. The great merit of Ostwald's classification is that it recognises the existence of the colloidal condition in any medium and for any physical state.

Defining the colloid substance as the disperse phase [2] and the continuous medium holding it in the colloidal condition as the dispersion medium, and recognising that the majority of colloid systems are di-phasic, we have by pairing the three physical states of matter, Ostwald's classification as follows :—

TABLE I. OSTWALD'S CLASSIFICATION OF COLLOID SYSTEMS.

Disperse Phase.	Dispersion Medium.	Abbreviation.	Example.
Solid.	Solid.	S/S	Gold in ruby glass.
Solid.	Liquid.	S/L	Metals, starch in water.
Solid.	Gas.	S/G	Smoke. Cosmic dust.
Liquid.	Solid.	L/S	Occlusions in minerals.
Liquid.	Liquid.	L/L	Emulsions.
Liquid.	Gas.	L/G	Mist, fog, clouds.
Gas.	Solid.	G/S	Pumice : lava.
Gas.	Liquid.	G/L	Foam.

For solid-in-liquid or liquid-in-liquid systems, Ostwald distinguished between those which are coarsely-dispersed (macroscopically or microscopically resolvable) and those colloidally dispersed (requiring an ultramicroscope for resolution).[3] The transition from one dispersion to another is not abrupt, but continuous, just as it passes continuously to the molecularly-dispersed state (true solution). Thus :—

TABLE 2.

Disperse Systems, Type S/L and L/L			
	Coarse Dispersions.	Colloidal "Solutions."	Molecular Solutions.
Diameter of Particles	$>0{\cdot}1\mu$	Between $0{\cdot}1\mu$ and $1{\cdot}0\mu\mu$	$<1{\cdot}0\mu\mu$

[1] *Vide* "A Handbook of Colloid Chemistry," by Wo. Ostwald, translated by Fischer (London, 1919), pp. 42 *seq.*

[2] *Kolloid-Z.*, **1**, 291 (1907).

[3] The limits for resolution are about $250\mu\mu$ for the microscope and $2\mu\mu$ for the ultramicroscope.

Since colloidal solutions represent a transition stage between coarse dispersions and molecular solutions, it follows that two general methods obtain for their preparation : (*a*) condensation from the molecular state ; (*b*) dispersion from coarser systems. Thus, colloidal solutions are possible because of : (1) the inherent size of the molecules of the colloid substance ; (2) aggregation of the elementary molecules.[1] The latter aspect is important in considering certain proteins.

INTERFACE PHENOMENA

The fact that in the transition from a molecularly-dispersed system to a coarse suspension the predominance of kinetic energy is diminishing and the predominance of surface effects is increasing, and the fact that when a coarse suspension is more finely dispersed, the surface exposed by the particles increases rapidly, led Alexander [2] to postulate a zone of maximum colloidality where the kinetic and surface effects are nearly balanced.

Considering colloid systems generally, the outstanding characteristic is that there exists a great separating area or boundary between the two phases, referred to hereafter as the interfacial area. Such an extended interfacial area calls into play surface forces, and it is along the lines of investigation into surface effects that colloid chemistry has made most notable progress in recent years. Although the term colloid chemistry is in general use, investigators have sought to find a description suggesting the enormous importance of surface effects. Thus, Freundlich : " The science of the physical properties of interfaces as connected with their material properties is called *capillary chemistry*." The term " surface " chemistry is in some use, although the expression more correctly would be " chemistry of interfaces." The most modern tendency is to avoid the word chemistry and to speak of " colloid physics."

The phenomena related to interfacial forces have been investigated thermodynamically by Willard Gibbs, Thomson, van der Waals and others, whilst the work of Hardy, Langmuir, Harkins Adam and others has been concerned with the question of the molecular structure of interfaces and the kinetics of reactions occurring there. Modern colloid chemistry or physics centres mainly round phenomena at interfaces, involving questions of structure and degree of dispersion. Evidently it must embrace the study of drops, bubbles, films, emulsions, gels, proteins, muds and slimes, foam, cells (animal and vegetable structures), catalysis, enzyme phenomena, and so forth.

[1] *Vide* Svedberg, " Colloid Chemistry " (New York, 1928), p. 150, where many references are given.

[2] *J. Am. Chem. Soc.*, **43,** 434 (1920) ; *Kolloid-Z.*, **36,** 334 (1925) ; " Colloid Chemistry," **1,** 21 (New York, 1926).

CHAPTER II

AGAR-AGAR AND GUMS

The United States of America Pharmacopœia defines agar-agar as "the dried mucilaginous substance extracted from . . . species of *Gelidium* and closely related algæ. Agar contains not more than 1 per cent. of foreign organic matter and yields not more than 1 per cent. acid-insoluble ash and not more than 16 per cent. moisture." Agar is practically nitrogen-free and is akin to the carbohydrates; being non-amphoteric its physico-chemical behaviour is radically different from that of gelatin. A typical analysis [1] of American agar is :—

	Per cent.
Moisture	18·41
Protein	1·12
N_2-free extract	76·94
Ether extract	0·19
Crude fibre	0·14
Ash	3·20

The ash contains : SO_3 (1·03 per cent.), P_2O_5 (0·01), Cl (0·01) SiO_2 (0·93), CaO (0·77), MgO (0·44), MnO (0·01), Fe, Al (0·04).

The agar of commerce is sold either as dried strands or powder, and is white or gray in colour. It is insoluble in cold water, but with boiling in water it yields a solution which, in so low a concentration as 0·75 per cent., sets on cooling to a rigid gel. Such gels exhibit marked syneresis.

In the food industries agar finds many uses ; in soups, jellies, pharmaceutical emulsions, sherbet and water ices, ice-cream, meat and fish pastes, and has even been recommended [2] for use in cheese-making. It is also employed in the canning of fish for transport of fragile fish in jelly, in the clarification of liquids, as a stabiliser in malted milk drinks, and in bread to inhibit staling. Its incorporation in breakfast and health foods, as well as in pharmaceutical preparations, depends on it being able to act mechanically (cf. cellulose of vegetable foods) as roughage which stimulates peristalsis. Undoubtedly its use is on the increase, and as it is widely used in bacteriological technique and is a non-protein colloid more and more

[1] *Chem. Met. Eng.*, **34,** 294 (1927). Numerous analyses are given by : Fellers, *Ind. Eng. Chem.*, **8,** 1128 (1916) ; Newcomb and Smythe, *J. Am. Pharm. Assoc.*, **10,** 524 (1921) ; Loeper *et al*, *Compt. rend. soc. biol.*, **79,** 660 (1916).

[2] Dahlberg, *J. Dairy Sci.*, **10,** 114 (1927).

used in colloid research, an account of its properties and behaviour becomes necessary.

PREPARATION OF AGAR

The preparation of agar has been described by Fellers,[1] Tressler,[2] Robertson [3] and Mackinnon.[4] The latter outlines the method used by the American Agar Co. at San Diego, California. The *Gelidium* species of seaweed is washed in water for one day and the agar then extracted by digestion with hot water under pressure. The solution is filtered hot with the aid of charcoal and Filter-Cel. On leaving the filter press, the hot liquor enters a storage tank and is then passed to a congealing machine, which it leaves as small rods of jelly. These rods are now frozen to form blocks of frozen agar of about 300 lbs. weight and containing 3 to 6 lbs. dried agar. Ice separates as crystals, whereupon the blocks are crushed and thawed. The mixture of ice-water and agar flakes thus obtained is filtered by a rotary vacuum filter, the agar being washed on the filter screen to remove soluble impurities. The residual flakes contain about 90 per cent. water and are dried in aluminium cylinders by a warm air blast.

Numerous patents [5] cover methods designed to remove impurities from agar. Utsuki [6] proposes to wash the seaweed and then treat for 1—2 hours in warm water containing 0·2—0·5 per cent. Na_2CO_3 or Na_2SO_4. The seaweed is then bleached with chlorine, sodium peroxide or hydrogen peroxide and boiled in water. The next operations are successively : filtration, concentration of filtrate *in vacuo*, freezing, crushing, thawing, separation and drying.

Prolonged soaking of agar in water at room temperature removed soluble matter, as illustrated by Mackinnon's data for Japanese and American agars :—

Time Soaked. Days.	Per cent. Loss in Dry Substance.	
	Japanese.	American.
6	40·8	22·8
10	46·3	27·1
14	48·5	28·3
21	50·0	30·5
33	51·8	32·0
47	53·0	33·2

[1] *Ind. Eng. Chem.*, **8**, 1128 (1916).
[2] " Marine Products of Commerce " (New York, 1923), p. 127.
[3] *Ind. Eng. Chem.*, 22, 1074 (1930).
[4] *Food Inds.*, **2**, 123 (1930).
[5] Cf. Steinitzer, German Pat. 269,088 (1912) ; Merck, German Pat., 272,145 (1913) ; Mimford, Brit. Pat. 134,011 (1918) ; Matsuoka, U.S. Pat. 1,399,359 (1921), and 1,453,848 (1923).
[6] Utsuki, Jap. Pat., 42,126 (1922).

CHEMICAL NATURE OF AGAR

Agar contains combined sulphur [1] and calcium, but whilst an agar sol contains calcium ions it does not contain sulphate ions.[2] Samec and Ssajevič [3] found that dialysis would not reduce the ash below 1·31 per cent. and that on heating dialysed agar to 120° C., some dialysable SO_4 could be recognised. They believed agar to be a gelose sulphate ("Agaropektin") with possibly silicate, and that 1 gr. atom of S corresponds to 8,900 gr. of a compound of formula $(C_6H_{10}O_5)_{54}SO_4H$.

Fairbrother and Mastin found that on hydrolysis with dilute HCl, almost all the sulphur content of agar appears as sulphuric acid. Even heating an agar hydrosol will reveal SO_4 ions, probably because of hydrolysis, thus :—

$$2\ H_2O + (R.O.SO_2.O)_2\ Ca \rightleftharpoons 2\ ROH + CaSO_4 + H_2SO_4.$$

The hydrolysis occurs slowly even in distilled water at room temperature, but is very rapid in dilute acid, *e.g.*, 2 per cent. agar sol containing HCl to 0·01N concentration, on heating for thirty minutes at 100° C. will no longer gel on cooling and shows the presence of free H_2SO_4. They showed that other metals may replace the calcium of agar without impairment of setting to gels, an observation recently confirmed by de Waele,[4] who finds that one can replace the SO_4 or Ca group, the former by Cl or NO_3, and the latter by any cation, and still obtain gels. According to de Waele, at pH < 2, hydrogen replaces calcium and no gel will form, but subsequent neutralisation with hydroxides, alkaloids, or aniline replaces hydrogen and restores the gel capacity. Heating an agar sol of reaction pH 2 causes hydrolysis, which produces simple glycides, reducing Fehling's solution and giving a positive furfural reaction.

The free acid, $R.O.SO_2.OH$, was obtained by Fairbrother and Mastin by alternate soaking of agar in 0·01N.HCl and water, to give an almost ash-free gel. On melting this gel hydrolysis occurs and cooling fails to restore the gel condition. Such hydrolysis is formulated as :—

$$(R.O.SO_2.O)_2\ Ca + 2\ HCl \rightleftharpoons 2\ R.O.SO_2.OH + CaCl_2.$$

Discussing the constitution of the agar gel these authors consider that "in the neutral state, the micellæ consist of undissociated salt and sulphuric ester ion, both possibly polymerised,

$$[H_2O]_x\ [(R.O.S_2O)_2\ Ca]_y\ [R.O.SO_2.O']_z$$

The Ca ions exist in the intermicellar spaces."

[1] Neuberg and Ohle, *Biochem. Z.*, **125,** 311 (1921).
[2] Fairbrother and Mastin, *J. Chem.* Soc., **123,** 1412 (1923).
[3] *Compt. rend.*, **173,** 1474 (1921); *Kolloidchem. Beihefte,* **16,** 285 (1922). Cf. Pauli and Valkó, "Elektrochemie der Kolloide" (Vienna, 1929), p. 608.
[4] *Ann. physiol. physicochem. biol.*, **5,** 877 (1929). See also Hoffman and Gortner, *J. Biol. Chem.*, **56,** 545 (1923).

Lüdtke [1] has recently discussed the constitution of agar. The sulphuric acid hydrolysate of agar contains levulinic acid, in amount increasing with the time of hydrolysis. This may come from galactose, and it may be present in a bound condition in the molecule. He believes that the acidity of an agar sol is due to this acid and the organically bound sulphuric acid.

HYDRATION OF AGAR

When placed in water or aqueous solutions agar swells and absorbs considerable amounts of water. The time factor is important as the following data by Fairbrother and Mastin show in tests carried out at 20° C :—

TABLE 3. VELOCITY OF SWELLING OF AGAR

Water Absorbed as per cent. of Dry Agar.

Days Elapsed.	0·25	1	2	3	4	7	14	29
H_2O . .	760	1,034	1,179	1,304	1,362	1,530	1,690	1,778
0·01 N.HCl .	467	523	525	535	544	553	558	565
0·01 N.NaOH.	661	760	808	855	887	940	1,009	1,085
1 : 500 Acetic Acid . .	490	607	671	706	723	744	789	812

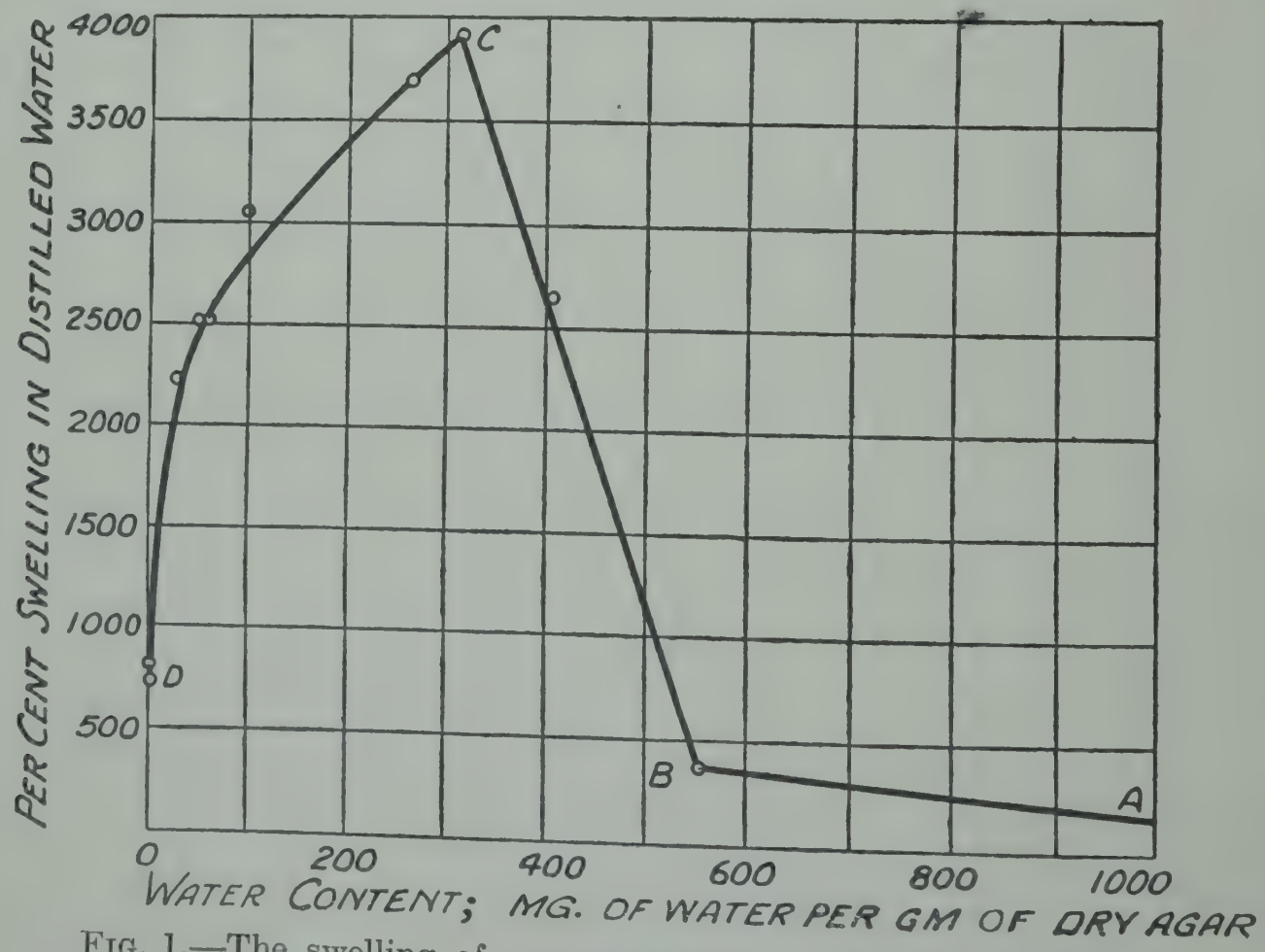


FIG. 1.—The swelling of agar-agar gels as a function of water content before swelling.

Agar may lose water by natural drying or by artificial desiccation. When such dried agar is placed in water, it is natural to suppose

[1] *Biochem. Z.*, **212**, 419 (1929).

that the drier the agar the more water it will take up. However, this is *not* the case, as Clarke [1] found in his investigation of the swelling of agar as a function of initial water content. His graph of the results obtained is reproduced in Fig. 1, and shows a sudden inflexion at B and a sharp maximum at C. It may be significant that B marks also the transition point of optical properties, fresh agar gels being opaque, but on drying changing abruptly to clear transparency. Perhaps the results depend on a change in gel structure.

Prát [2] has found a similar effect, dry agar giving less swelling than wet agar in 0·12—0·164 M.KCl, NaCl, and $CaCl_2$. No marked catonic differential was noticed.[3] The additive effects of $Na^{\cdot}$, $K^{\cdot}$ and $Ca^{\cdot\cdot}$ were peculiar : $K^{\cdot} + Ca^{\cdot\cdot}$ or $Na^{\cdot} + Ca^{\cdot\cdot}$ produced an increase in swelling much exceeding the single salt effect, whilst $K^{\cdot} + Na^{\cdot} + Ca^{\cdot\cdot}$ produced less swelling than in two salt combinations or in pure single-salt solutions.

Considerable research has been conducted on the swelling of agar under the influence of electrolytes.[4] Michaelis [5] finds that the swelling of agar jellies is greatest in pure water, the addition of any electrolyte reducing swelling. He differentiates between two concentration ranges of added electrolytes : (A) up to 0·1N. wherein the anion, irrespective of valency, is of no importance, whilst the cation is subject to a valence effect, the hydrogen ion, however, being more effective than trivalent cations ; (B) 0·1N—1N, wherein there is an anionic effect and the cations follow the order of a lyotropic series, *i.e.*, arranged according to their hydration capacity for water. In the last instance, the effect is due to those ions which are *not* combined with or adsorbed by the agar. In (A) all the effects depend on electric charge.

Dokan's [6] experiments support these conclusions. Electrolytes may influence colloidal dispersion in two ways : (1) reduction of the electric charge, in a measure due to the valency of the ion bearing the sign opposite to that of the colloid particles ; (2) a lyotropic dehydration effect, a measure of the relative hydration capacity of the colloid particles and the ions.[7] Studying the swelling of agar in solutions of electrolytes, Dokan observed no lyotropic dehydration up to 0·1N. concentration, but thereafter, as the concentration increased, the Hofmeister or lyotropic ion series became more pro-

[1] *J. Am. Chem. Soc.*, **47,** 1954 (1925).
[2] *Am. J. Botany,* **14,** 167 (1927).
[3] This is contrary to MacDougall, *Science,* **62,** 136 (1925).
[4] Cf. MacDougall, *Carnegie Inst. Year Book,* **20,** 55 (1921) ; *Proc. Am. Phil. Soc.*, **63,** 76 (1924).
[5] *Am. Coll. Symp. Monog.*, 2, 13 (1925) ; Michaelis, " The Effect of Ions in Colloidal Systems " (Baltimore, 1925), pp. 88 *et seq.* See also Iwase, *J. Tokyo Chem. Soc.*, **41,** 468 (1920), and Buchner and Kleijn, *Proc. Acad. Sci., Amsterdam,* **30,** 740 (1927).
[6] *Kolloid-Z.*, **34,** 155 (1924).
[7] *Vide* Freundlich " Colloid and Capillary Chemistry " (London, 1926), pp. 427, 576, 833.

nounced.[1] Thus, for chlorides, the cationic series was La<Na<K, whilst for potassium salts the anionic series was I< rhodanide <NO_3<Br<Cl< acetate <SO_4< citrate.

The OH ion behaves as any other monovalent anion, and when its concentration is such that lyotropic dehydration becomes evident, it falls between Br and SCN in the series. Hydrogen ions, on the other hand, are very effective in low concentrations, and are even stronger than trivalent cations.

As illustrating the magnitude of the effects obtained, the following table is reproduced from Dokan's paper :—

TABLE 4. SWELLING OF AGAR IN 0·002 EQUIVALENT NORMAL SOLUTIONS OF CHLORIDES AFTER 24 HOURS.

Chloride.	Percentage Swelling.
Water . .	100·00
KCl . .	82·22
NaCl . .	74·84
LiCl . .	73·40
$MgCl_2$ . .	50·63
$CaCl_2$ . .	49·18
$BaCl_2$ . .	45·85
$AlCl_3$ . .	35·42

Working with a 2·2 per cent. (purified) *agar gel*, de Jong [2] observed that immersion of such gel in distilled water at room temperature resulted, not in swelling, but in a slow shrinkage or syneresis. All neutral electrolytes in dilute solution caused a slight shrinkage of the gel, the maximum linear contraction being 1 per cent. Non-electrolytes such as sucrose or isoamyl alcohol were without influence. This contraction in the region of dilute electrolytes is a capillary-electric phenomenon influenced solely by the valency of the cation and independent of the nature of the cation or the valency and nature of the anion. Sexavalent cations exert their effect in extremely dilute solutions, the maximum contraction of the gel occurring in solutions so dilute as 1·5 milli-equivalents per litre.

It was found that the electric discharge type of contraction is reversible, the volume of the gel increasing when the electrolyte was washed out again. Alcohol in high concentrations produces a linear contraction of the gel to the extent of 7 per cent., but is reversible. Its action is attributed to dehydration of the agar.

[1] Cf. similar results by Iwase, *loc. cit.*
[2] *Kolloidchem. Beihefte*, **29**, 454 (1929).

Hydration shrinkage can arise with high concentration of electrolyte. Thus, $MgSO_4$ causes greater contraction as its concentration increases because of its dehydrating capacity, whereas $MgCl_2$ up to 5N. concentration does not produce any greater contraction than its weaker solutions, since it is less able to dehydrate the gel particles.

SWELLING OF AGAR IN ACIDS

Fairbrother and Mastin [1] report that acids decrease the swelling of agar to a degree practically in linear relation to the pH value of the solution. Thus, swelling in HCl, H_2SO_4, oxalic, acetic and phosphoric acids is practically the same at the same pH value. On the other hand, in very dilute NaOH, agar swells more than it does in water alone, but in more concentrated NaOH the swelling is inferior to that in water. The following table (p. 12), abridged from the authors' data, illustrates the magnitude of the swelling of agar in systems of interest to the food chemist and the bacteriologist.

ELECTRODIALYSIS OF AGAR HYDROSOLS

Several investigators have resorted to electrodialysis for the special purification of agar solutions for research purposes. Ordinary dialysis fails to remove all the ash.[2] Employing shredded U.S. Pharm. agar, Harvey [3] washed this twice in 0·5 per cent. acetic acid and then in six changes of distilled water over a period of 12 hours. A hot solution of 100 gr. agar in 3,000 c.c. water was then prepared and electrodialysis conducted at 50° C. for 12 hours, by which time the sol had separated into a flocculent lower layer and a clear upper layer. The latter was siphoned off, filtered through paper and super-centrifuged at 40,000 r.p.m. The clear transparent liquor, which showed very quick setting at room temperature, was rapidly dried at 40° C., and the solid, which resembled sheet gelatin, had an ash content of 0·81 per cent. as compared with the original value of 3·75 per cent. The pH value of a hot 1 per cent. sol of the purified agar was 4·90.

The agar was found by cataphoresis to be negatively charged. The viscosity of a 1 per cent. sol, determined at 45° C., varied with the pH value, reaching a maximum at pH 7. Thus, the relative viscosity at pH 2·3 and pH 10·7 was 1·16, but at pH 7·1 it reached 1·26.

Hoffman and Gortner [4] have also described the electrodialysis of agar, employing Bacto Agar of moisture content 11·39 per cent.

[1] *Loc. cit.*
[2] Samec and Ssajevič, *Compt. rend.*, **173,** 1474 (1921).
[3] *Am. J. Pharm.*, **97,** 66 (1925).
[4] *J. Biol. Chem.*, **65,** 371 (1925).

Table 5. The Swelling of Agar in relation to pH.

(R.S. = relative swelling, *i.e.*, compared with absorption of water from pure water, reckoned as 100).

Approximate Initial Concn.	H_2O	$\frac{N}{25600}$	$\frac{N}{12800}$	$\frac{N}{6400}$	$\frac{N}{3200}$	$\frac{N}{1600}$	$\frac{N}{800}$	$\frac{N}{400}$	$\frac{N}{100}$	$\frac{N}{50}$	$\frac{N}{25}$	$\frac{N}{12{\cdot}5}$	$\frac{N}{6{\cdot}25}$
Hydrochloric Acid :													
pH . . .	5·3	4·6	4·0	3·6	3·3	—	2·9	2·6	2·0	1·7	1·4	1·1	0·9
R.S. . . .	100	99·0	78·5	74·5	63·0	—	57·0	53·5	42·5	36·0	32·0	30·5	29·5
Acetic Acid :													
pH . . .	5·6	4·9	4·5	4·2	4·0	3·8	3·6	3·5	3·1	3·05	3·0	2·9	—
R.S. . . .	100	83·0	87·0	77·5	77·0	69·5	69·5	64·5	62·5	62·5	62·0	64·5	—
Phosphoric Acid :													
pH . . .	5·5	5·0	4·8	4·6	4·0	3·6	3·3	3·0	2·6	2·3	2·1	1·9	—
R.S. . . .	100	91·0	97·5	91·5	83·0	69·0	69·0	64·5	60·0	56·5	54·0	45·5	—
Sodium Hydroxide :													
pH . . .	5·5	6·2	8·4	9·8	—	10·4	11·3	11·8	12·1	12·3	12·6	12·8	—
R.S. . . .	100	112·5	118·5	129·0	125·0	112·5	—	98·5	79·5	69·0	64·0	64·0	—

The Foster-Schmidt [1] apparatus was used, this being a three-compartment box, each of 1,250 c.c. capacity, separated by heavy sheets of collodion supported by a double layer of light canvas. The electrodes were carbon plates (12 × 15 cm.) and the voltage applied was 220 D.C.

Fifty gr. coarse-ground, air-dried agar were suspended in 1,250 c.c. distilled water in the middle compartment. The end compartments each held 1,250 c.c. distilled water, replaced hourly. Electrodialysis progressed for 18 hours, when the granules of agar became very swollen and translucent and their aqueous suspension gave a pH value of 2·0. The liquid surrounding the agar granules contained 0·7 per cent. dry matter, apparently in solution, and proved a remarkably effective protective colloid for the preparation of metal hydrosols.

The agar was filtered off and dried at 30° C. A 5 per cent. solution was only slightly viscous and showed no tendency to gelatinise. Simple heating with water led to considerable hydrolysis with the appearance of reducing sugars. The analyses before and after electrodialysis are given below, the figures being on the basis of the original dried agar :—

	Normal Agar. Per cent.	Purified Agar. Per cent.
Ash	4·66 ..	0·70
Sulphur . . .	1·01 ..	1·01
CaO	0·99 ..	0·04
SiO_2	0·53 ..	0·57

There has evidently been an almost quantitative removal of calcium and the data support the view that agar is the calcium salt of a sulphuric acid ester, $R.O.SO_2.OH$.

HYDROSOLS AND GELS OF AGAR

Increasing interest is being given to the study of agar sols and gels in aqueous medium, particularly in respect to their optical properties. Hatschek and Humphry [2] prepared *clear* sols up to 7 per cent. agar content by holding the solutions at 80° C. for 2—4 hours, whereby the insoluble constituents gradually unite to a dirty grey coagulum, readily filtered out by glass wool.[3]

Such sols containing 3 per cent. or more agar differ in optical properties from the corresponding gels, the sols being clear in transmitted and reflected light, whereas the gels show clear in

[1] *Ibid.*, **56,** 545 (1923).
[2] *Trans. Faraday Soc.*, **20,** 18 (1924).
[3] Cf. Kruyt and de Jong, *Kolloidchem. Beihefte,* **28,** 9 (1928), who flocculate at 50° C. and use filter paper.

transmitted light, but exhibit pronounced opalescence in reflected light. Due to the lateral scattering of light, the gels appear darker in transmitted light than the corresponding sols. Ageing the gels for several days at 62° C. (the approximate mean between setting and melting points) does not decrease the opalescence of the gels.

It was further observed that the electrolytic conductivity of a sol containing electrolyte ($CuSO_4$ in M/12·5 concentration) is less than that of the corresponding gel system, determinations being made at 50° C. The difference in conductivity increases as the agar concentration increases. No proven explanation of this peculiar behaviour is yet forthcoming, although the results have been confirmed and extended by Iwase.[1]

In an extended investigation, Donnan and Krishnamurti [2] studied the scattering of light by agar sols and gels, such scattering increasing with increase in the number and size of the scattering particles (Rayleigh's law).

Working with a 1 per cent. agar sol over the temperature range 80° C.—25° C., the authors observed no change in the light scattering until about 35° C.; below this temperature the light scattering increases rapidly.[3] Thus, there is an increase in the number and/or size of the colloid aggregates. It is significant that this increase precedes the sol-gel transformation and continues even when the system sets to a firm gel.

When the gel is heated to about 90° C. it reverts to a sol which possesses the same light scattering capacity as the original sol. No appreciable change in light scattering is apparent on heating the gel until about 40° C. is reached, and the experimental data show that although up to about 90° C. the gel remains quite firm, there is a progressive diminution in bulk of the colloidal micelles constituting the gel. It would seem that as the temperature is elevated " more and more of the material constituting the gel micelles passes into the intermicellar liquid, until finally the structure or framework constituting the gel disappears and we obtain a sol which may be polydisperse, and may contain a large proportion of the agar in a molecularly or nearly molecularly disperse form."

Because of hysteresis it is possible to examine the light-scattering capacity of both agar sols and gels over a considerable temperature range. Experimental determinations at temperatures from 40° C.—70° C. for 1 per cent. agar systems indicate that the gel scatters more light than the sol, the difference being less as the temperature increases. Thus, the following values are given for I (intensity of scattered light) in 1 per cent. agar :—

[1] *Bull. Chem. Soc. Japan*, **2**, 61 (1927).
[2] *Proc. Roy. Soc.*, **A 122**, 76 (1929); *Am. Coll. Symp. Monog.*, **7**, 1 (1930).
[3] Cf. de Jong, *Rec. trav. chim.*, **47**, 797 (1928), who found 35° C. to be of particular importance in viscosity/time curves for agar sols.

Temperature.	1 (Sol).	1 (Gel).	$\frac{1\ (Gel).}{1\ (Sol).}$
40° C.	10·9	107·4	9·9
50° C.	10·6	97·7	9·2
60° C.	10·6	88·0	8·3
70° C.	10·5	73·5	7·0

Donnan and Krishnamurti point out that possibly the agar is molecularly dispersed (or nearly so) in the sols, but in the gels the agar is present as relatively large aggregates or micelles. They refer to Hatschek's alternative suggestion that the high values of I (gel) may arise from the fact "that gelation is due to the segregation of a phase much richer and an aqueous phase much poorer in agar than the sol. Such an action would produce a considerable difference in the refractive indices of the gel particles and the intermicellar liquid." Hatschek [1] observed that the graph obtained by plotting I (gel) against concentration of gel gave a maximum at about 13 per cent. agar. He believes that hydration is not the only factor concerned in the increase in Tyndall effect when agar sol sets to a gel, but that pronounced hydration of the particles leads to an increase in their size. Due to the hydration of the agar, the total volume of the system, agar + water, is reduced and thereby the refractive index is raised.

Increasing the concentration of agar increases the value I (sol), but even in 2 per cent. sols the value is only 19·0 at 35° C., whereas in the case of I (gel) the concentration function is marked and the value 220 is reached for 2 per cent. agar over the temperature range 20°—50° C. The concentrated gels obviously contain numerous and large colloid particles.

The main point of interest in this work is the recognition that the cooling of an agar sol leads to a great increase in the number of micelles before gelation sets in,[2] and that reduction in temperature causes a marked increase in the total volume of these micelles long after the firm elastic gel has been obtained. Conversely, raising the temperature of the gel leads to a progressive diminution of the total volume of the micelles, such diminution commencing and progressing continuously long before that temperature is attained, which marks the passing of the gel into the sol condition. Hence it is evident that "increases and decreases of the 'colloidality' of agar sols and gels occur continuously with continuous variation of temperature and are subject to no abrupt or discontinuous changes corresponding to the usually recognised sol→gel and gel→sol transitions."

[1] *Kolloid-Z.*, **48,** 246 (1929).
[2] Cf. de Jong, *Rec. trav. chim.*, **47,** 797 (1928).

PORE SIZE OF AGAR GELS

Whilst Voigtlander [1] found that several electrolytes diffused into agar gels at 20° C. independently of the concentration of the gel, Bechold and Ziegler [2] observed that red beet juice diffused more slowly as the gel concentration increased. Recently, Friedman [3] has reported that as the gel concentration increased from 0·8 per cent. to 5·15 per cent., the diffusion of urea slowed down 36 per cent. Glycerin diffusion was also slowed down.

Friedman's investigation resulted in the following values for the radii of the " pores " in agar gels :—

Agar in Gel (per cent.).	Diffusate.	
	Urea.	Glycerin.
2	2·9 mμ	5·65 mμ
5	0·74 mμ	1·43 mμ

The pores are smaller than in corresponding gelatin gels. Glycerin has a pronounced influence on agar and its presence greatly accelerates the rate of diffusion of urea into the gel. That various non-electrolytes do influence gel structure is evidenced, too, by the remarkable effect of dextrose and trimethylene glycol in accelerating the diffusion of urea into agar gels, whilst ethyl alcohol retards such diffusion.

AGAR AS A LYOPHILIC COLLOID

Important investigations by Kruyt and his co-workers have led to a decided clarification of ideas regarding the colloid behaviour and the stability factors of agar hydrosols. For the student of general colloid chemistry these newer conceptions demand consideration. As viscosity measurements play no small part in this connection, the viscosity factor will serve as a convenient starting point in the discussion.

The viscosity of an agar sol is reduced by heating, *e.g.*, the relative viscosity of a 1 per cent. sol (measured at 45° C.) is 1·16 on just boiling and 1·035 after 30 minutes' boiling.[4] It is also influenced by the presence of electrolytes.[5]

Kruyt and de Jong[6] have presented a detailed report on the

[1] *Z. physik. Chem.*, **3,** 316 (1889).
[2] *Ibid.*, **56,** 105 (1906).
[3] *J. Am. Chem. Soc.*, **52,** 1311 (1930).
[4] Harvey, *Am. J. Pharm.*, **97,** 447 (1925).
[5] Harvey, *Ibid.*, **97,** 66 (1925).
[6] *Kolloidchem. Beihefte,* **28,** 1 (1928). See Kruyt, " Colloids " (New York, 1930), Chap. 13, for a full discussion.

influence of electrolytes on the viscosity of agar sols. Einstein's theoretical formula for the viscosity of a liquid system containing in dispersed form incompressible particles is :—

$$\eta_s = \eta_0 (1 + \frac{5}{2}\phi)$$

where η_s = viscosity of the system.
η_0 = ,, ,, liquid medium.
ϕ = volume of the particles as a fraction of the total volume.

Now $\frac{\eta_s}{\eta_0} - 1 = \frac{5}{2}\phi$

where $\frac{\eta_s}{\eta_0}$ = relative viscosity of the sol as compared with the medium.

Thus $\frac{\eta_s}{\eta_0} - 1 = \frac{\eta_s - \eta_0}{\eta_0}$ = increase in *relative* viscosity due to the disperse phase (and thus proportional to the volume of the disperse phase).

With emulsoids, where the definition of incompressibility may fail, we find high viscosities at even low concentrations. Thus, Kruyt and de Jong observed for a 0·279 per cent. agar sol at 50° C. a relative viscosity of 2·392, or about 200 times the calculated value. Such abnormal values arising from the greatly increased volume of the agar particles may be explained by (*a*) hydration of the particles, or (*b*) sponge-like aggregation of the particles. Hydration plays the predominant *rôle* in the case of agar. The magnitude of the viscosities is illustrated by the following data from the authors' paper :—

RELATIVE VISCOSITY OF AGAR SOLS

Agar Content.	40° C.	45° C.	50° C.
0·071 per cent.	1·330	1·325	1·322
0·143 ,,	1·674	1·662	1·652
0·285 ,,	2·419	2·393	2·364

The viscosity is strongly reduced by all electrolytes in dilute concentration, and KCl, NaCl, LiCl, NH_4Cl, KCNS, K_2SO_4 and $K_4Fe(CN)_6$ in equivalent concentrations produce practically the same reduction in viscosity. A similar but greater decrease is effected by $BaCl_2$, $SrCl_2$, $MgSO_4$ and $CdSO_4$. The valency of the cation determines the magnitude of the salt effect. Trivalent and

quadrivalent salts exert a still greater influence. Fig. 2, reproduced from the authors' paper, illustrates the influence of electrolytes on viscosity. The decrease in viscosity soon subsides to a constant value and the authors believe this is due to the effect of the electric discharge of the agar particles.[1] Agar was found to be negatively charged and von Smoluchowski has shown on theoretical grounds

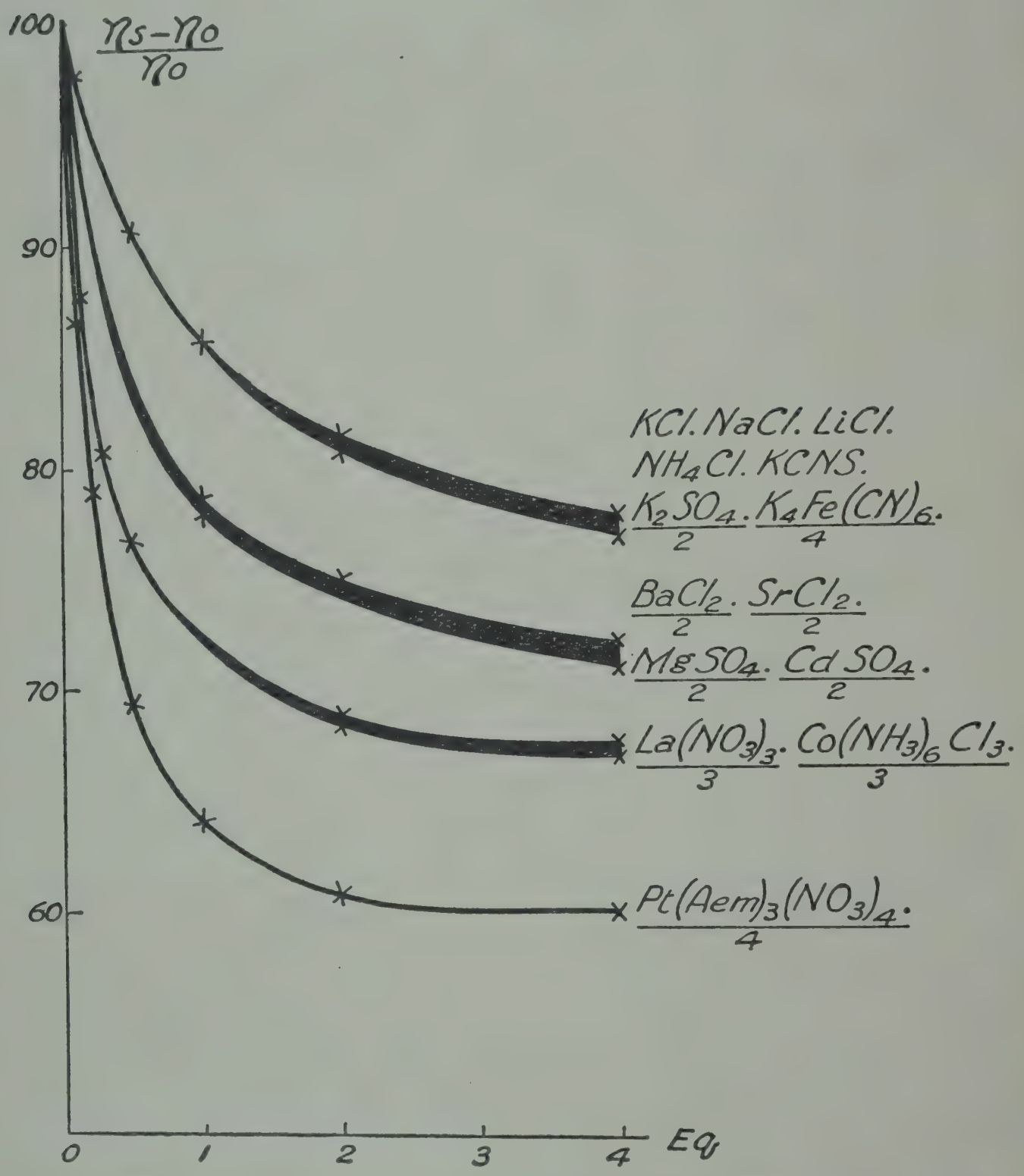


FIG. 2.—The electro-viscous effect illustrated for an agar-agar sol. The valence of the cation is quite noticeable, whilst that of the anion is very small. The experimentally determined curves for the monovalent cations fall within the upper black band; those for the divalent cations, within the second band; and so on. Abscissæ represent milliequivalents.

that the viscosity of a charged suspensoid must be greater than that of the uncharged.

The reduction in viscosity due to addition of HCl or NaOH is greater than that by $BaCl_2$. Again, organic ions are as a rule more strongly adsorbed by agar than are inorganic ions and therefore

[1] Cf. Kruyt and de Jong, *Z. physik. Chem.*, **35**, 250 (1922).

fuschin, for example, produces a greater decrease in viscosity than does KCl.

With greater concentration of electrolyte, where the electroviscous effect is absent, the relative decrease in viscosity is a function of the hydration of the ions and this increases over the lyotropic series : $K_2SO_4 < KCl < KNO_3 < KCNS$.

When an agar sol is diluted with alcohol or acetone at 50° C. and then cooled, the ultramicroscope shows numerous bright particles in vigorous Brownian motion, whereas similar dilution

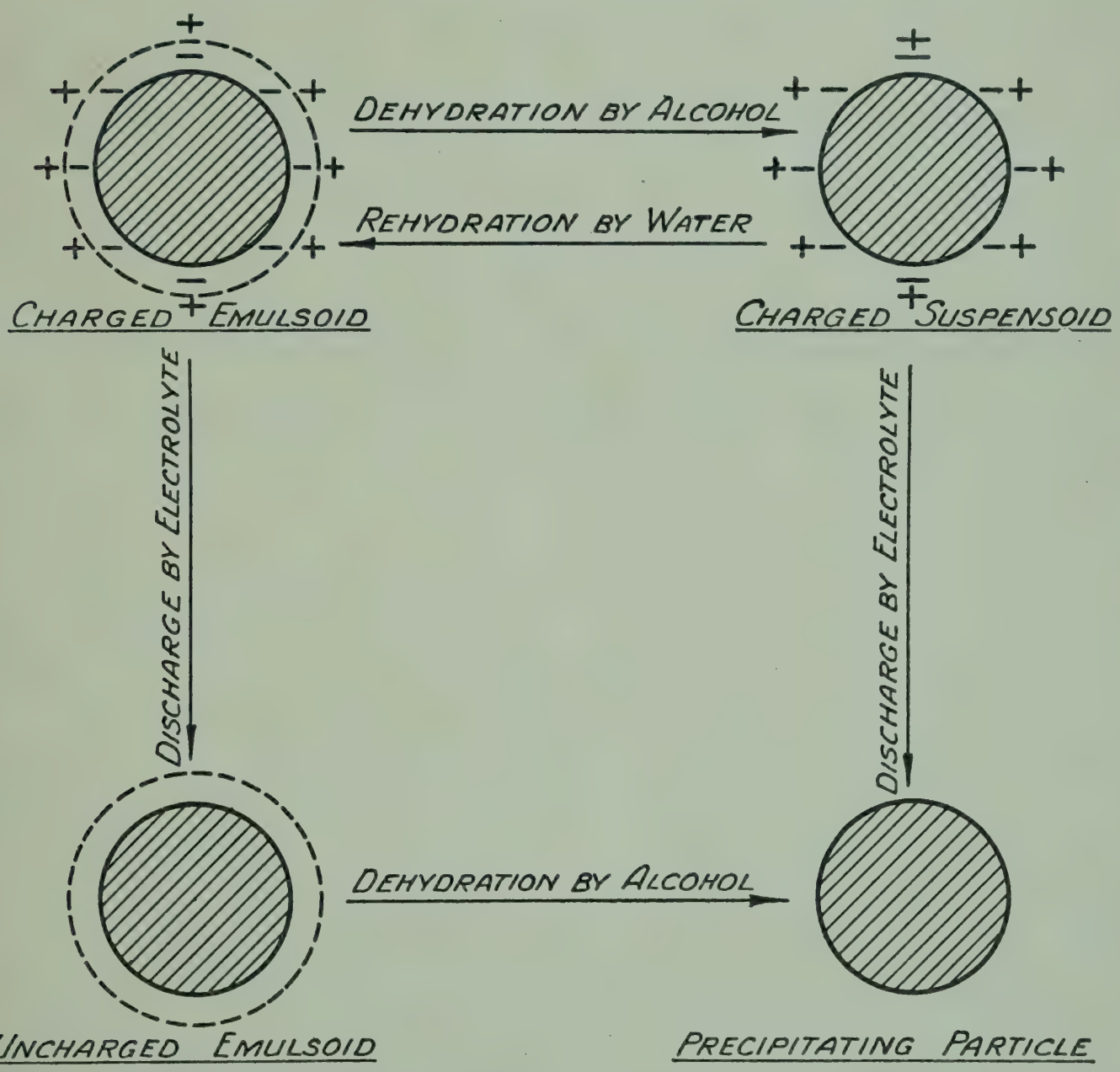


FIG. 3.—Kruyt's diagram to illustrate the stability of an emulsoid sol of the simplest type, such as one of agar-agar or starch.

with water only shows a Tyndall cone. Dehydration of the agar has transformed an emulsoid system into a suspensoid system, which is now sensitive to the influence of electrolytes. Alternatively, if one removes the electric charge from an agar sol by addition of minute amounts of electrolytes, further addition of alcohol will bring about immediate flocculation. Thus, there are two stability factors involved in an agar sol, the removal of both being necessary to produce flocculation.

Emulsoids in general can be "salted out" or precipitated by large quantities of such electrolytes as $MgSO_4$ and Na_2SO_4. Both the nature of the cation and of the anion play a part in this

phenomenon, the cation causing electric discharge of the agar particles and the anion acting as a dehydrating agent, hence the anion lyotropic series effect : $SO_4 < Cl < Br < NO_3 < I < CNS$. Kruyt and de Jong picture the argument in Fig. 3, where, it will be noted, the dehydration of the electrically-charged particle is reversible, while this is not the case in the removal of the electric charge. Again, the dehydration of the uncharged agar is an irreversible flocculation. Kruyt has extended the above considerations to a generalised conception of the stability of colloids, whether suspensoids or emulsoids.

AGAR IN BACTERIOLOGICAL TECHNIQUE

Since agar gives gels which remain solid at 37° C. it finds wide application in routine bacteriology. The usual preparation of " nutrient agar " is to add 2 per cent. of agar powder or 2·5 per cent. of shredded agar to " broth " and heat in the steam steriliser for 1 hour. The solution is then cooled to 55° C. and the whites of two eggs beaten in to each litre. Then follows steaming for 2 hours, or autoclave digestion at 5 lbs. pressure for 1 hour, when the solution is filtered hot through Chardin paper. This medium melts at 98° C. but does not reset before 42° C.

The purification of agar for bacteriological purposes has been proposed by various workers. Thus, Cunningham [1] soaks agar for 10 minutes in 0·01 per cent. H_2SO_4 and then washes thoroughly with water. After filtration the filtrate is partially dried and then heated at 120° C. for 1 hour. The product is then dried at moderate temperature. This agar in 1 per cent. concentration gives a firm gel. Fellers [2] purifies agar by washing in dilute acetic acid and then in water and precipitating a hot 5 per cent. sol by alcohol or acetone. Such treatment removes much of the nitrogenous matter of the agar, which is important since the nitrogenous constituents are available for bacteria,[3] and one frequently requires a jellying medium with a minimum of nutrients. Other methods for removing nitrogen are described by Klostermann [4] and Pekelharing.[5] The latter worker found that holding an agar sol at 50° C. for 24 hours before filtering, resulted in a nitrogen decrease from 1·6 per cent. to 0·39 per cent.

Peptone is a usual ingredient in culture media, and Fellers [2] reports that this substance increases the jellying power of agar. Agar gels show pronounced syneresis, an objection in bacteriological

[1] *Indian J. Med. Research*, **5,** 560 (1919).
[2] *Ind. Eng. Chem.*, **8,** 1128 (1916).
[3] Fellers, *Soil Science*, **2,** 257 (1916).
[4] *Z. Hyg. Infektionskrankh.*, **94,** 262 (1921).
[5] *Proc. K. Akad. Wetensch., Amsterdam,* **30,** 309 (1922). See also : Hardy, *Z. physik. Chem.*, **33,** 326 (1900) ; Kruyt and de Jong, *Kolloidchem. Beihefte*, **28** 6 (1928) ; de Jong, *Ibid.*, **29,** 458 (1929).

routine, particularly with agar slants. Moreover, the exudate is not simply water but a nutrient solution. Its composition has been discussed in relation to bacteriological routine by Pijper[1] and Healey.[2]

IRISH MOSS

Irish moss or *Chondrus crispus*, Lyngb., is a member of the Gigartinaceæ, belonging to the Floridæ or red seaweeds. It is a perennial plant, attaining maximum development in spring and summer. The weed is sold in commerce as the yellowish-brown fronds, mixed with *Gigartina mamillosa*. The powder form is now common. Its nitrogen content is low, from 2—3 per cent., while the ash content may reach as high as 15 per cent.

In the food industries, Irish moss is used as a thickening agent in blanc-manges and jellies, as a fining agent[3] and as an agent in preparing emulsions. Haas and his co-workers[4] have made a special study of the colloidal character of the moss. Extraction with hot water yields 70—75 per cent. soluble matter, whilst cold water extraction only gave 47 per cent. soluble matter in 36 days. The hot water extract enabled the preparation of very stable emulsions of cod-liver oil emulsions, whereas the cold water extract produced unstable emulsions. Again, Rochelle salt profoundly assists in the setting of a 1 per cent. solution of a hot water extract but not the cold. Hence there are two colloidal ingredients, one of which is more soluble in cold water than the other.

Gels can be prepared by soaking the moss in water and warming to 60° C. On cooling a 1 per cent. solution gives a gel, a 3 per cent. solution a fairly stiff gel, and a 5 per cent. solution a good gel. The melting point of the 3 per cent. gel is 27°—30° C. and that of the 5 per cent. gel is 40°—41° C. The gelatinising power is retained even after long boiling, *e.g.*, a 3 per cent. solution after $3\frac{1}{2}$ hours' reflux boiling sets on cooling.

Gutbier and Huber[5] have discussed the general colloid character of Irish moss extract preparatory to their investigation of its use as a protective colloid for metal hydrosols. By dialysis of a 0·56 per cent. solution for 22 days, the ash content was reduced to 0·02 per cent. The solutions were found to be stable against microbiological action, but ageing resulted in decreased viscosity. Thus a 0·55 per cent. mucilage kept at 25° C. gave the following viscosity values relative to water as unity :—

Age in days . .	0	1	2	5	15	33
Viscosity . .	15·29	14·87	14·11	10·52	7·74	7·19

[1] *S. Afr. Med. Record*, **17,** 372 (1919).
[2] *J. Bact.*, **12,** 179 (1926).
[3] Rankin, *J. Inst. Brewing*, **35,** 287 (1929).
[4] *Pharm. J.*, **106,** 485 (1921) ; *Ann. Appl. Biol.*, **7,** 352 (1921) ; *Biochem. J.*, **15,** 469 (1921) ; **16,** 265 (1922) ; *Analyst*, **52,** 265 (1927).
[5] *Kolloid-Z.*, **30,** 20 (1922).

A decrease in viscosity was also noted on heating. Thus for a 0·49 per cent. mucilage boiled for different times :—

Mins. boiled . .	5	15	25	45	75	135	165
Viscosity . .	6·89	6·43	6·19	5·80	5·47	5·01	5·01

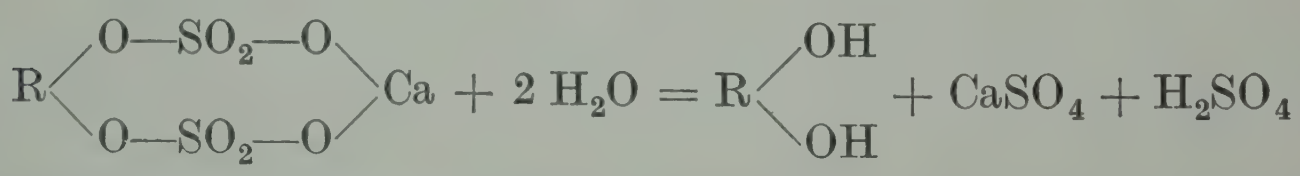
The marked increase in viscosity as the concentration of the solution was increased is seen in the following data :—

Concentration (per cent.)	1·07	0·89	0·76	0·67	0·59	0·45	0·36	0·18
Viscosity (25° C.) .	36·96	31·17	18·18	14·15	11·71	9·02	7·39	4·45

The addition of electrolytes reduced the viscosity of the solution until a constant value was reached, at about 0·12 N. for HCl, NaOH and NaCl. A more extensive investigation of the action of electrolytes on viscosity has been conducted by de Jong and Gwan,[1] who find the viscosity extraordinarily sensitive to electrolytes even in traces. As little as 1 milliequivalent of electrolyte causes pronounced decrease in viscosity, the valency of the cation being the important factor. This research has interest in connection with the theory of the electro-viscous effect in colloid systems.[2]

Heating Irish moss with acid rapidly effects loss of gelatinising power,[3] a point deserving attention in any project to use the substance as a thickening agent in acetic sauces.

An aqueous solution of the moss shows the presence of Ca ions but not of SO_4 ions, but after hydrolysis the latter ions appear in the solution, due to the decomposition of an ethereal sulphate. The calcium can be quantitatively precipitated from solution. The work of Haas has led him to assume the existence of a complex able to liberate SO_4 ions after hydrolysis. Thus :—



Quantitative analyses have proved the correctness of this formula.

Harwood [4] has examined Irish moss as a colloidal electrolyte, accepting the above formula. He points out that the calculated molecular weight is about 1000, whereas the colloidal behaviour of Irish moss shows its complexity to be much greater than this low value indicates. The formula would suggest that in solution there would be pronounced ionisation producing ionic micelles. Determinations of the electrical conductivity of the solutions over a range of dilutions have confirmed this view and the conductivity at

[1] *Kolloidchem. Beihefte.*, **29,** 436 (1929).
[2] Kruyt, " Colloids " (New York, 1930), pp. 183–85.
[3] Haas and Russell-Wells, *Biochem. J.*, **23,** 425 (1929).
[4] *J. Chem. Soc.*, **123,** 2254 (1923).

infinite dilution is of the same order as that of Ca. Hence the colloidal ion must possess a mobility very similar to that of SO_4 ion. From Donnan membrane equilibrium studies the experimental results by Harwood strongly support the view that Irish moss extract is a calcium salt of an organic acid in which the SO_4 group is contained in the complex radicle R.

GUM ARABIC

Gum arabic finds application chiefly in pharmaceutical emulsions and is seldom used in such foodstuffs as sauces or ketchups, as its thickening power is low except in concentrated dispersions, and even then the presence of acids or salts is detrimental to the desired effect.

First grade gum arabic or Hashab gum is obtained from *Acacia Senegal*,[1] but the usual commercial article is frequently a mixture of gums from various plants.[2] The average analysis gives 15 per cent. water and 3·5 per cent. ash, mainly K_2CO_3 and $CaCO_3$. The first exudate of gum is not entirely soluble in water, but after 2 or 3 months' storage it becomes completely soluble due to enzyme action.[3] The gum functions as an acid and is considered to be constituted from arabic acid associated with galactan and araban.

The preparation and the general chemistry of gum arabic are described by Stocks,[4] Garner [5] and Trotman.[6] The gum is a protective colloid [7] of molecular weight 1,750, and its gold number varies from 0·5 to 4·0. Its solubility in water has been determined by Taft and Malm [8] who used gum purified by precipitation by alcohol from aqueous solution. They found that 1 part of gum dissolved in 1·5, 1·6, and 1·7 parts of water at 90° C., 50° C. and 25° C. respectively.

Gum arabic lowers the surface tension of water, as witness the following table, due to Clark and Mann [9] :—

SURFACE TENSION OF GUM ARABIC SOLUTIONS AT 25° C.

Concentration.	Surface Tension.
0·1 per cent.	72·30 dynes/cm.
0·5 ,, ...	72·18
1·0 ,, ...	72·04
5·0 ,, ...	69·69
10·0 ,, ...	61·49

[1] Cf. Ridgway, *Can. Chem. J.*, **4,** 330 (1920).
[2] Cf. Alland, *Schweiz. Apoth-Ztg.*, **53,** 31 (1915).
[3] *Vide Farben-Ztg.*, **25,** 960 (1920).
[4] *Brit. Assocn. Coll. Repts.*, **1,** 53 (1917).
[5] *Ind. Chemist.*, **3,** 341 (1927).
[6] *Chem. Trade J.*, **82,** 500, 525, 601 (1928).
[7] Boutaric, J. *chim. phys.*, **25,** 120 (1928); cf. Voigt, *Kolloid-Z.*, **54,** 307 (1931).
[8] *Trans. Kansas Acad. Sci.*, **32,** 49 (1929).
[9] *J. Biol. Chem.*, **52,** 178 (1922); cf. Walter, *Sitzb. Akad. Wiss. Wien.*, Abt. II.A., **129,** 709 (1920).

The gum is not adsorbed from aqueous solution by animal charcoal, but after 48 hours' contact with $Al(OH)_3$ about 44 per cent. of the gum is irreversibly adsorbed. This leads Rakuzin [1] to the view that an aqueous solution of gum arabic contains two constituents, an electrolyte adsorbable by $Al(OH)_3$, and a non-adsorbable non-electrolyte. Confirmation of this is required.

Kruyt and Tendeloo [2] have investigated the viscosity of gum arabic solutions and find Poiseuille's law to be obeyed. The following are their results at 25° C., showing viscosity as a function of the concentration of the gum :—

Concentration.	Relative Viscosity.
0·0311 per cent.	1·060
0·0622 ,,	1·104
0·1244 ,,	1·185
0·2490 ,,	1·321
0·4983 ,,	1·564
1·0000 ,,	2·015

Clark and Mann's figures for the same temperature show that the relative viscosity of a 0·1 per cent. solution is 1·115, and of 10 per cent. solution is 8·781.

Electrolytes appreciably reduce the viscosity.[3] Kruyt and Tendeloo observed that the greatest lowering occurs with the initial small additions of electrolyte. Thereafter, higher concentration of electrolyte brings about partial dehydration of the sol, as proved by the ultramicroscope. When there is a combination of dehydration and electric discharge, as by the addition of alcohol and an electrolyte *e.g.*, acetic acid, the gum is flocculated.

Recently an important investigation has been made by Thomas and Murray,[4] which throws light on the problem of the constitution and colloidal behaviour of gum arabic. They improved on the older methods of purification, such as precipitation of the gum by alcohol (concentration $>$ 60 per cent.) or by dialysis,[5] by employing electrodialysis. One hundred gr. of gum were stirred into 500 c.c. water containing 5 c.c. of 12 N.HCl, and after 30 minutes complete precipitation was effected with alcohol. After filtration through linen, this cycle of operations was repeated four times. The gum was now dissolved in water and subjected to electrodialysis in a three-compartment wooden cell having collodion membranes, until free from chlorine, the solution being adjusted to reaction

[1] *J. Russ. Phys. Chem. Soc.*, **53,** 357 (1921).

[2] *Kolloidchem. Beihefte.*, **29,** 396 (1929). *Vide* also, Walter, *loc. cit.*, and Clark and Mann, *loc. cit.*

[3] Dhar and Chakravarti, Alexander's "Colloid Chemistry," Vol. 2, 114 (New York, 1928).

[4] *J. Phys. Chem.*, **32,** 676 (1928).

[5] Graham, *J. Chem. Soc.*, **15,** 257 (1862), reduced the ash by dialysis of 20 per cent. gum in 4—5 per cent. HCl.

pH 5 with ammonium hydrate. The temperature was never permitted to exceed 40° C.

ARABIC ACID

In this way a purified arabic acid was obtained which did not reduce Fehling's solution, in contradistinction to the original gum. A 1 per cent. solution of the acid had a reaction of pH 2·70, corresponding to a strong acid. The ash content of the arabic acid was only 0·08 per cent. (original value 2·78 per cent.), but the nitrogen figure only fell to 0·33 per cent. (original value 0·36 per cent.). It proved necessary to keep the acid in aqueous solution, since drying or precipitation by alcohol leads to a product which is practically insoluble in water.

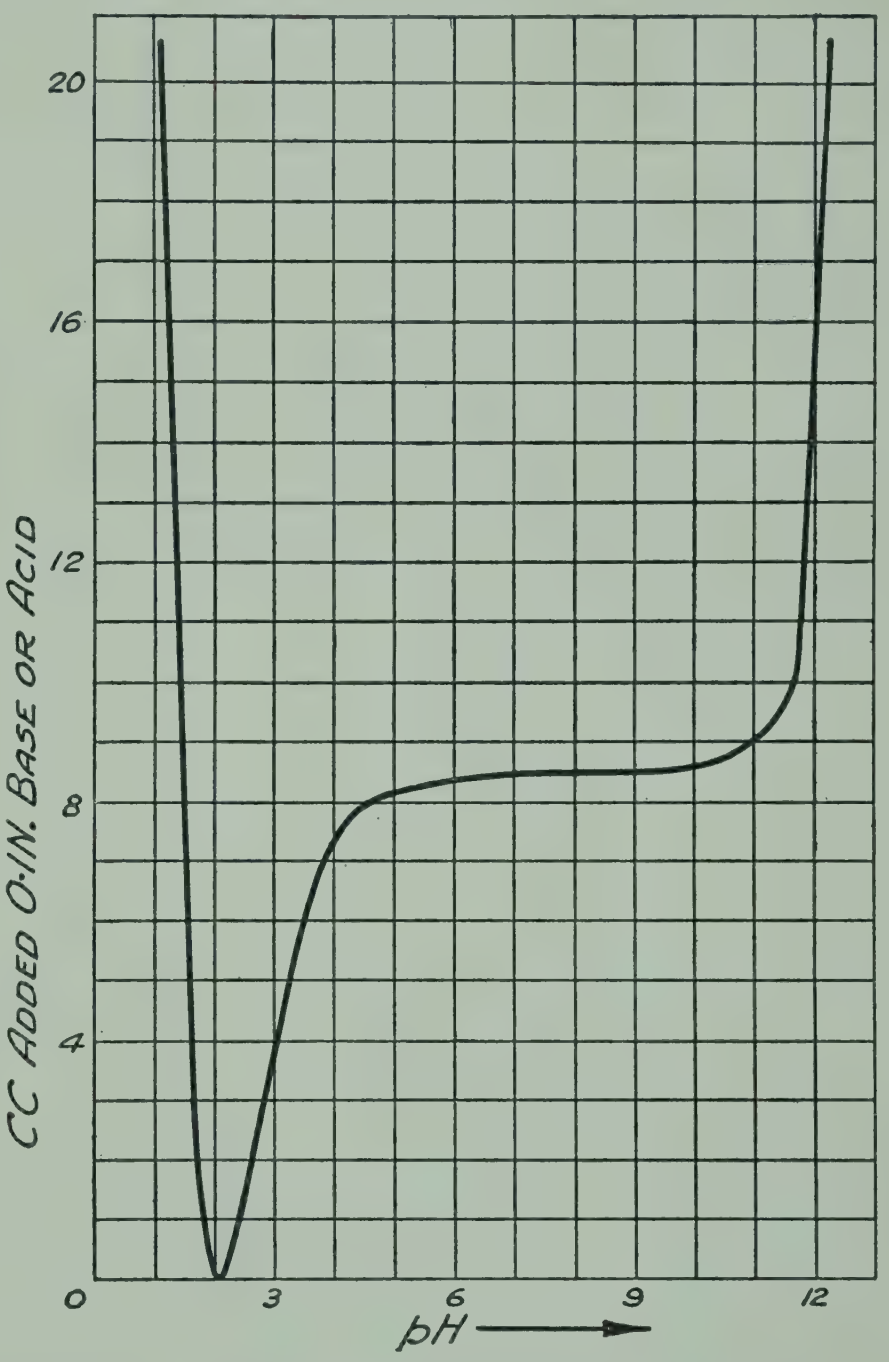


Fig. 4.—Titration of arabic acid with acid and alkali.

The titration curve of arabic acid is reproduced in Fig. 4. One gr. portions in 15—20 c.c. solution were titrated electrometrically with 0·1N.NaOH, $Ba(OH)_2$ and HCl. Since the curves for the two alkalies are practically identical, the acid combines with alkali in equivalent proportions according to the laws of primary valency. A region of neutrality exists over the zone pH 5—10. The sharp inflexion indicates a strong acid character. Arabic acid is not amphoteric and its equivalent weight [1] is calculated as 1,177.

Thomas and Murray determined the viscosity of arabic acid as a function of pH, using the Ostwald viscometer at 25° C. for 1 per cent. solutions. By addition of HCl or NaOH, the pH range,

[1] Cf. Amy, *Bull. soc. chim. biol.*, **10,** 1079 (1928), who also electrodialysed gum arabic and obtained the complex monovalent organic acid (dissociation constant = approx. 2×10^{-4}) of molecular weight 1600.

0·74—13·33 was explored. The viscosity showed a maximum at pH 4·58—6·3 (see Fig. 5, reproduced from the authors' paper). The presence of neutral salts depressed the viscosity in a measure increasing with their concentration. Such viscosity behaviours were attributed to the existence of the arabic acid in the form of micelles, and the colloidal behaviour generally was found to accord with the qualitative application of Donnan's theory of Membrane Equilibrium.

Recent research by Taft and Malm [1] has confirmed and extended

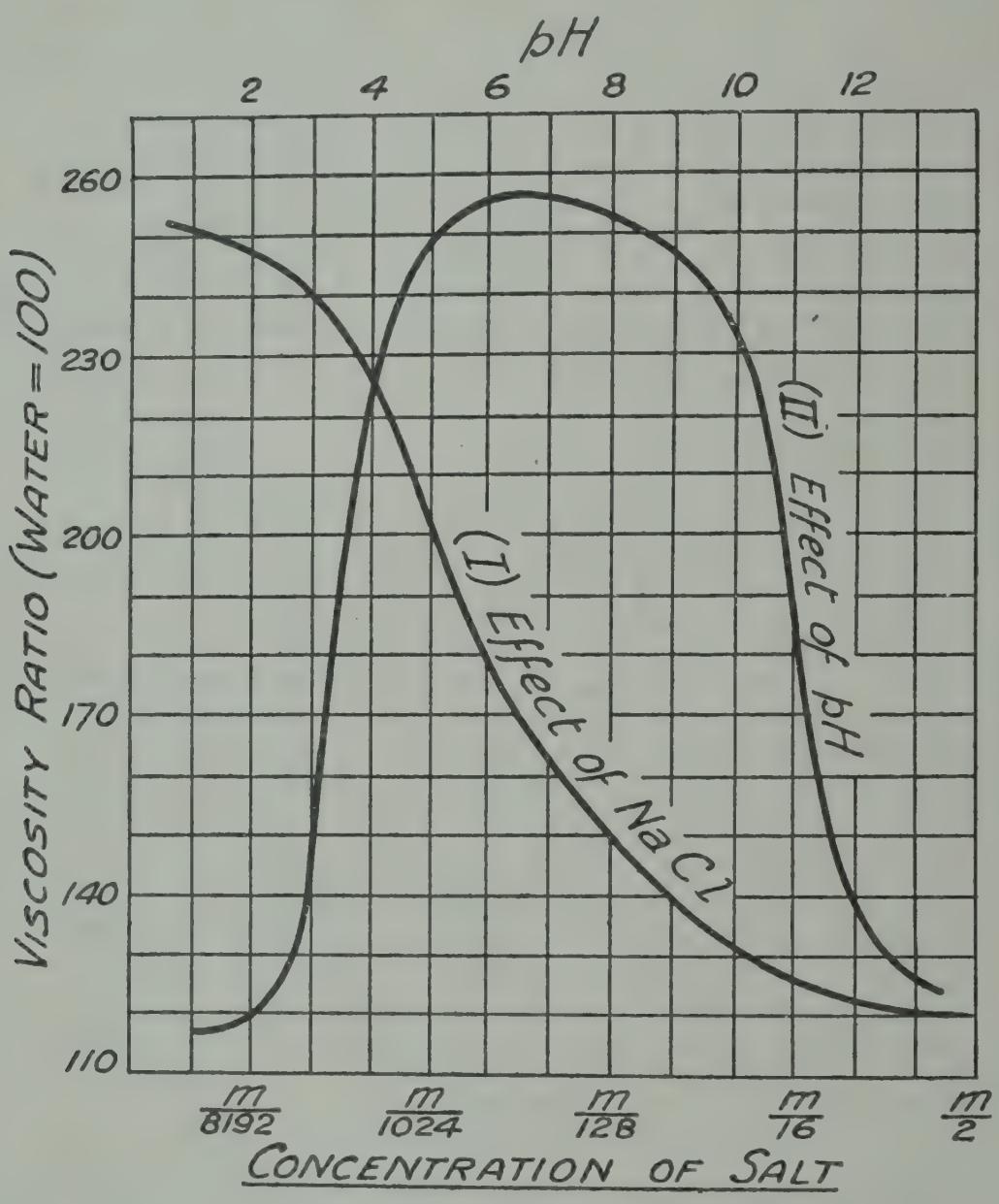


Fig. 5.—Viscosity of gum arabic solution as a function of pH and of neutral salt concentration.

this work, the authors carrying out measurements of viscosity, density, freezing point and electrical conductivity of gum arabic-water systems with variation in concentration and temperature. They believe that " purified gum arabic is a strong organic electrolyte of high equivalent weight, rather than existing as a colloidal phase when in contact with water."

GUM TRAGACANTH

This gum is used as a thickener in sauces and as an emulsifying agent in certain pharmaceutical emulsions. It is an incision product from Asiatic low spiny shrubs of the genus *Astralgus*.

[1] *J. Phys. Chem.*, **35,** 874 (1931).

The literature relating to the colloid chemistry of gum tragacanth is meagre.[1] The gum consists of two main fractions, arabin and bassorin, in approximately equal amounts. Arabin is soluble in water, whilst bassorin is not soluble. The former yields a typical hydrosol, the latter swells to a gel. At 5 per cent. concentration, gum tragacanth is a very thick mucilage, and for its proper dispersion for use in food products the mucilage is passed through a colloid mill.

Gutbier and his associates [2] have described some of the properties of gum tragacanth mucilage which they employed as a protective colloid for certain metal hydrosols. Its gold number is about 2.[3] On standing, a solution of the gum will in a few days show a sediment, and the system loses its viscosity. Heating results in a rapid diminution in viscosity. Thus, Gutbier examined a 0·2 per cent. dialysed solution of the gum and observed the following viscosities at different temperatures :—

Temperature .	15° C.	20° C.	30° C.	40° C.	50° C.	60° C.	70° C.	80° C.
Viscosity. .	7·3	6·4	5	4	3·26	2·65	2·17	1·64

The viscosity is also reduced by the addition of acid, alkali, and NaCl, particularly on heating.

Tragacanth appears to exert a pronounced buffer action between pH 3 and pH 10, as the following table by Krantz indicates.[4] Weak gels (0·5 per cent. gum) were prepared in water adjusted to different pH values.

TABLE 6. BUFFER ACTION IN TRAGACANTH GELS

pH of Solvent.	pH of Gel.	pH of Solvent.	pH of Gel.
0·04	0·04	6·48	5·88
0·95	0·96	7·86	5·82
1·92	2·10	9·75	7·70
2·88	3·81	10·47	9·99
3·93	4·93	11·85	11·50
4·78	5·54	12·74	12·68
5·80	5·56	13·56	13·65

GUM TRAGON

Gum tragon [5] or locust-kernel gum has lately found considerable incorporation in sauces and ketchups, replacing the more expensive gum tragacanth. It is marketed as a white powder, which is

[1] *Vide* Stocks, *loc. cit.*, p. 57.
[2] *Kolloid-Z.*, **18,** 141 (1916) ; **32,** 331 (1923).
[3] Morse, in the writer's laboratory.
[4] *J. Am. Pharm. Assocn.*, **18,** 471 (1929) ; **19**, 1181 (1930).
[5] Casaburn and Simonsini, *Collegium* (**1924**), 137.

obtained by the hot water extraction of the endosperm of the carob bean [1] (*ceratonia siliqua*), otherwise known as the locust bean or St. John's bread. The extract is filtered, dried and ground and several patents [2] cover technical modifications of this simple procedure.

The gum in aqueous solution is a negatively-charged colloid,[3] the gold number as determined by Morse [4] being 0·15. Even in a concentration of 0·5 per cent. the aqueous solution is thick and viscous, whilst a 5 per cent. solution on cooling sets to a stiff gel. Very little work has been reported on the colloid chemistry of gum tragon, although its dialysis and use as a protective colloid for metal hydrosols have been investigated.[5]

Williams [6] has examined the gum, which is a carbohydrate, $C_6H_{10}O_5$, precipitatable from its aqueous solution by alcohol and by basic salts, *e.g.*, basic lead acetate. Acids lower the viscosity, particularly on heating, a fact of importance when it is desired to use the gum in sauces. The viscosity is raised by alkali and the colour darkens.

A characteristic reaction with tannin serves to distinguish the gum from gum tragacanth. Excess of tannin added to the aqueous solution causes a soft gelatinous mass to separate, marked syneresis of the gel later leading to two phases : a light-coloured clot and a clear supernatant liquor. On heating to 40°—50° C. the system changes to a homogeneous, very viscous and turbid mucilage in the case of concentrated solutions and a clear liquid in the case of dilute solutions. On cooling, the system reverts to its original two-phase character. It is interesting to note that the tannin-gum complex is redispersed by alkalies, glycerol or sugar.

CHEWING GUM

The article of confectionery known as chewing gum is made from various ingredients, principally *chicle*, which has largely replaced the original spruce and cherry gums. The subject has some interest for the colloid chemist,[7] since the chewing gum is a brittle solid which on mastication must yield a highly plastic mass capable of being abnormally extended.

[1] For analytical data see :—Balland, *Compt. rend. acad. agr. France*, **4,** 969 (1918) ; Hodgson, *Analyst*, **46,** 366 (1921) ; Gouin, *J. agr. prakt.*, **82,** 388 (1918) ; Walton, U.S. Dept. Agr., *Bull.*, **1194,** 1—19 (1923) ; Oddo, *Chimie et industrie*, **20,** 207 (1928) ; Jaffa and Albro, Calif. Agr. Expt. Sta., *Bull.* **309,** 441 (1919).

[2] Audibert, U.S. Pat., 1,593,544 (1926) ; Pinel, *Ibid.*, 1,106,355 (1914) ; Windgassen, French Pat., 663,802 (1928) ; Müller, U.S. Pat., 1,749,833 (1930) ; Produit Melassés, German Pat., 501,407 (1927).

[3] Locher, *et al.*, *Kolloid-Z.*, **33,** 37 (1923).

[4] In the writer's laboratory.

[5] Gutbier *et al.*, *Kolloid-Z.*, **33,** 37 (1923) ; **34,** 336 (1924).

[6] *Analyst*, **53,** 411 (1928). See also Hart, *Ind. Eng. Chem., Anal. Edn.*, **2,** 329 (1930).

[7] Ditmar, *Gummi-Ztg.*, **40,** 156 (1925).

Chicle is the gum prepared from the sap of the tropical American tree *sapodilla*. Incisions into the bark permit collection of the sap, which is boiled to the desired consistency and moulded into blocks for export. The best grades are firm, almost white and practically tasteless. According to Dannerth [1] the blocks usually contain about 40 per cent. moisture. He gives the following analysis of a Yucatan chicle :—

Acetone-soluble (resins) . .	= 40·0	per cent.
Gutta (and carbohydrates) . .	= 17·4	,,
Proteins	= 0·6	,,
Sand and foreign matter . .	= 2·3	,,
Water	= 35·0	,,
Ash	= 4·7	,,

In making chewing gum the chicle is mixed with sugar and flavourings.[2] Weber [3] proposes mixing chicle with cumarone resin (M.Pt. 60° C.). Numerous patents cover the use of other plastic substances in place of chicle ; latex of the cow-tree (*Brosimum galactodendron*),[4] latex of *Dyera costulate*,[5] synthetic resins, *e.g.*, from a vinyl ester and an aldehyde,[6] resin ex Pontianak ext. or amarillo,[7] and rubber.[8]

Considerable scope for research is open on such questions as the relation of moisture and temperature to plasticity, and the elaboration of routine methods for testing the capacity of ingredients to serve in making suitable recipes. The degree of dispersion of the " tacky " ingredient would also seem to warrant investigation.

[1] *Ind. Eng. Chem.*, **9**, 679 (1917).
[2] Raub., U.S. Pat., 1,136,613 (1915).
[3] U.S. Pat., 1,402,817 (1922).
[4] Pratt, U.S. Pat., 1,240,875 (1917).
[5] Hamlin, U.S. Pat., 1,674,435 (1928).
[6] Matheson, Brit. Pat., 280,247 (1926).
[7] Bradshaw, U.S. Pat., 1,134,073 (1915).
[8] Beatty, U.S. Pat., 1,418,271 (1922). Cf. also Snyder, U.S. Pat., 1,239,313 (1917), and Meier, U.S. Pat., 1,171,392 (1916) for other recipes.

CHAPTER III

THE CHEMISTRY OF THE PROTEINS

The study of proteins is of the greatest importance to the food chemist from two points of view : (1) the physiological chemistry of protein foods, (2) the physico-chemical behaviour of proteins in solution and gel form. The scope of the present volume necessarily precludes discussion of the first point.

Proteins are condensation products of various amino-acids, the resulting large molecule bringing the substances within the range of colloid chemistry. Some workers believe that there is not a single large protein molecule, but rather varying complexes of high order.[1] Certain experiments by Shibata [2] support the view that polymerised amino-acids occur as anhydrides in the protein molecule.

Some typical molecular weight values [3] are collected in Table 7.

TABLE 7.

Protein.	Molecular Weight.	Observer.
Casein . . .	33,600 ± 250	Burk and Greenberg.[4]
Edestin . . .	(*a*) 49,500 ± 700	Burk and Greenberg.[4]
	(*b*) 228,000	Lamm.[5]
Egg-albumin . .	(*a*) 34,000	Sörensen.[6]
	(*b*) 34,500	Svedberg.[7]
	(*c*) 36,000	Burk and Greenberg.[4]
Hæmoglobin . .	(*a*) 34,300 ± 425	Burk and Greenberg.[4]
	(*b*) 66,000	Svedberg and Fahrens.[8]
Serum-albumin . .	(*a*) 5,345	Pauli and Schön.[9]
	(*b*) 66,167	De Caro and Laporta.[10]
	(*c*) 67,500	Svedberg.[7]
	(*d*) 74,000	Lamm.[5]
Serum-globulin . .	103,600	Svedberg.[7]

[1] Cf. Malfitano and Sigaud, *J. chim. phys.*, **24,** 259 (1927).
[2] *Acta Phytochimie*, **2,** iv, 193 (1927).
[3] *Vide*, Cohn, Hendry and Prentiss, *J. Biol. Chem.*, **63,** 721 (1925), for numerous values.
[4] *Ibid.*, **87,** 197 (1930).
[5] *Z. physik. Chem.*, **143,** 177 (1929).
[6] *Compt. rend. trav. lab. Carlsberg.*, **12,** 1—372 (1917).
[7] " Colloid Chemistry " (New York : 1928), p. 164.
[8] *J. Am. Chem. Soc.*, **48,** 430 (1926). *Vide* Adair, *Proc. Roy. Soc.*, **A 109,** 292 (1925).
[9] *Biochem. Z.*, **153,** 253 (1924).
[10] *Arch. sci. biol. Italy*, **14,** 264 (1930).

The remarkable variation in the values obtained by different investigators finds an explanation in the brilliant work by Svedberg [1] and his pupils on the stability of proteins as a function of the pH value of the system examined. His ultracentrifuge permits not only the determination of molecular weight but determines the homogeneity or otherwise of the protein with regard to molecular weight at any pH value. Thus, lactalbumin [2] has been found to be non-homogeneous as regards molecular weight. Values ranging from 12,000—25,000 were observed. It was concluded that in cows' milk is a material of low molecular weight (below 1,000), and the lactalbumin of high molecular weight is the result of the gradual aggregation of this material.

Certain proteins have been ascertained to be monodisperse and to fall into two groups : (A) Proteins with molecular weights from 34,500 to 208,000 ; (B) the hæmocyanins, with molecular weights of the order 10^6. Group (A) has four sub-groups, the molecular weights being 1, 2, 3 and 6 times 34,500 respectively. Svedberg gives the following table of molecular weights :—

TABLE 8. MOLECULAR WEIGHTS OF MONODISPERSE PROTEINS.

Group.	Class.	Protein.	Molecular Weight.
(A)	1	Ovalbumin . .	34,500 = 1·00 × 34,500
		Bence-Jones protein .	35,000 = 1·01 × 34,500
	2	Hæmoglobin . .	68,000 = 1·97 × 34,500
		Serum-albumin . .	67,500 = 1·96 × 34,500
	3	Serum-globulin . .	103,800 = 3·03 × 34,500
	4	Amandin . . .	208,000 = 6·04 × 34,500
		Edestin . . .	208,000 = 6·04 × 34,500
		Excelsin . . .	212,000 = 6·15 × 34,500
		Legumin . . .	208,000 = 6·04 × 34,500
		C — Phycocyan .	208,000 = 6·04 × 34,500
		R — Phycocyan .	206,000 = 5·97 × 34,500
		R — Phycoerythrin .	209,000 = 6·06 × 34·500
(B)		H — Hæmocyanin .	5×10^6
		L — Hæmocyanin .	2×10^6

The pH stability region of these proteins is fairly wide and includes the isoelectric point. Raising the pH over a certain value causes the proteins of group (A), classes 2, 3 and 4, to dissociate into molecules of a lower number of groups of 34,500. Thus, legumin, with molecular weight 208,000, is stable over the pH range 5—9. At a pH value approximately 9·3, there are present molecules of

[1] *Nature*, **123**, 871 (1929) ; *Kolloid-Z.*, **51**, 10 (1930) ; *Trans. Faraday Soc.*, **26**, 740 (1930) ; *J. Am. Chem. Soc.*, **52**, 2855, 2897, 3279 (1930).

[2] Sjögren and Svedberg, *J. Am. Chem. Soc.*, **52**, 3650 (1930).

one-half the normal weight, and still smaller units are formed if the pH is <5 or >9. All these proteins break down to units having the same molecular weight, *viz.*, 34,500, when the alkalinity is sufficiently high. Svedberg made the important observation, based on electrophoresis tests, that the integer molecules of a protein are electrochemically identical with the original molecule, whilst proteins of the same molecular weight, but chemically different, are also electrochemically different. Equally important, too, is the observation that : " If we continue to raise or to lower the pH of the solution we finally arrive at a point where the protein molecule is completely broken down into fragments of low molecular weight, and of an electrochemically different behaviour. This complete decomposition is in many cases reversible, *i.e.*, if the mixture is restored to a pH situated within the stability region, molecules of the original weight are built up again out of the fragments." Svedberg's work is bound to reflect deeply on the future development of the theory of the colloid chemistry of proteins.

PROTEINS AS AMPHOLYTES

Since amino-acids contain both NH_2 and COOH groups, proteins exhibit amphoteric reactions, *i.e.*, they are able under certain conditions to function either as bases or as acids. When the basic NH_2 group unites with an acid, such as HCl, a protein hydrochloride results, and when the acidic COOH group unites with a base, such as NaOH, a proteinate results, *e.g.*, sodium proteinate. Both types of compound undergo ionisation in solution ; the following scheme shows these ionisations, R representing the protein structure, which is united to the amino and carboxyl groups :—

$$R\begin{cases}NH_2\cdot HCl\\COOH\end{cases} \rightleftharpoons \left(R\begin{cases}NH_3\\COOH\end{cases}\right)^{+} + Cl^{-}$$

$$R\begin{cases}NH_2\\COONa\end{cases} \rightleftharpoons \left(R\begin{cases}NH_2\\COO\end{cases}\right)^{-} + Na^{+}$$

As the protein molecule is amphoteric, its behaviour in aqueous solution is profoundly influenced by the concentration of hydrogen or hydroxyl ions therein, *i.e.*, by the pH value of the solution. Thus, gelatin in contact with dilute HCl (or NaOH) goes into solution in proportion to the amount of HCl (or NaOH) present, acid (or alkali) disappearing from solution simultaneously. Solubility passes through a minimum in the case of acid but not in the case of alkali.[1]

The fact of minimum solubility of proteins at a definite hydrogen ion concentration was emphasised by Hardy,[2] who termed the point

[1] Fairbrother and Swan, *J. Chem. Soc.*, **121,** 1237 (1922).
[2] *J. Physiol.*, **24,** 288 (1899) ; **33,** 251 (1905).

the *isoelectric point*, since here the electric charge on the molecule is neither positive nor negative. Later work has shown that the isoelectric point is a physical constant of all proteins. At the isoelectric point the protein shows no preference for combination with acid or alkali. The original simple definition is now more complicated (see p. 46).

CLASSICAL VIEWS ON PROTEIN BEHAVIOUR

Before discussing more modern work, it is of interest to summarise the well-known views of Robertson.[1] Although proteins are agreed to react with acids and bases stoichiometrically, he pointed out that there is no agreement as to the mechanism of the interaction. Whether there is chemical union or physical adsorption of ions is really a matter of definition, and the colloid character of proteins does not necessarily depend on the existence of molecular aggregates, since the individual molecules themselves are large enough to come within the zone of colloidality.[2]

The first important point raised by Robertson is that it is quite incorrect to infer that neutralisation of a protein is due exclusively to the terminal COOH and NH_2 groups of the protein molecule. The amphoteric nature of proteins cannot be so simply discussed. As a matter of fact several investigators [3] have shown that of the total nitrogen in proteins only a very small fraction is present as free amino groups. As example, only 1·8 per cent. of the total nitrogen of edestin is present in union as free NH_2 groups, and as regards casein at least 50 per cent. of its capacity to neutralise acids is due to elements in the molecule other than NH_2 groups.

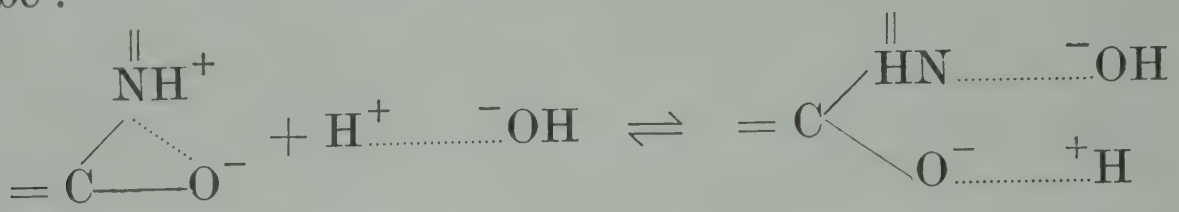
Robertson believed that the union of protein with acids or bases is also effected by means of the COHN groups within the protein molecule. In fact, the predominant method of protein combination with acids and bases is through the internal COHN groups, an idea supported by the experiments of Hitchcock [4] on the diminution of the acid binding capacity of deaminised gelatin (*i.e.*, treated with HNO_2).

Emil Fischer frequently suggested that diketopiperazine rings formed an important part in the building of protein molecules, and work in Robertson's laboratory confirmed this. Accepting such a structure, the union of the amino acids in the protein molecule would result in " the neutralisation of all of the amino groups in the constituent radicals of the protein molecule excepting those which are contained in diamino (lysyl) radicals."

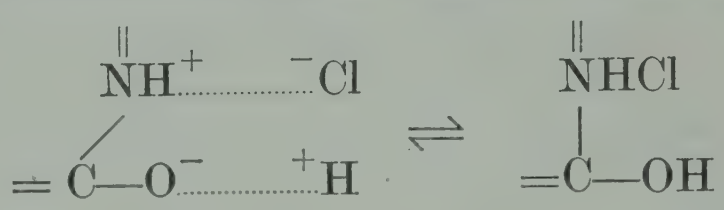
Robertson contended that condensation of the keto form of the

[1] " The Physical Chemistry of the Proteins " (New York, 1918).
[2] Cf. Lindestrøm-Lang, *Compt. rend. trav. lab. Carlsberg*, **15**, 1 (1924).
[3] Levites, *Z. physiol. Chem.*, **43**, 202 (1904); *Biochem. Z.*, **20**, 224 (1909); Van Slyke and Birchard, *J. Biol. Chem.*, **16**, 539 (1913).
[4] *J. Gen. Physiol.*, **6**, 95 (1923/24).

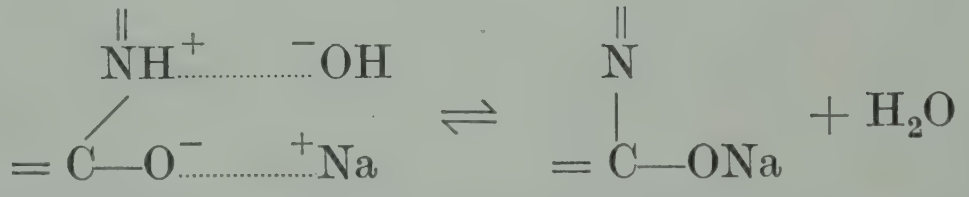
diketopiperazines would yield a compound whose properties would be those of neutral (isoelectric) protein. Its structure would be such that at each linkage there are residual valencies which, in the isoelectric protein, are internally neutralised. Should ions be present in the solution the orientation of some of the residual valencies towards the ions would confer acid or basic characters to the protein. In water, the equilibrium electronic orientation would be :—



In pure water the change of electronic orientation would be slight and depend on how far the isoelectric point is removed from pH 7. If excess of hydrogen ions be present, the charge would increase and, when sufficiently great, lead to chemical union.



With excess of hydroxyl ions, the protein combination with base would result :—



Using " adsorption " in the Langmuir sense as attraction due to residual valencies, Robertson agreed that adsorption is the first step in the formation of chemical compounds with proteins. After a detailed discussion of the ionisation of protein salts, he arrived at the conclusion that " the compounds of proteins with acids and alkalis are ionised in aqueous solution, but in many cases, for instance the caseinates and serum globulinates of the alkalis and alkaline earths, and the compound of egg-albumin or of albumose with hydrochloric acid, the inorganic ion is not dissociated as such. The ions which are actually present in these solutions must therefore be protein ions, a proportion of which carry the inorganic radical bound in a non-dissociable form."

Protein solutions exhibit certain peculiarities in relation to viscosity and Robertson dealt with this question on the lines that the protein molecule is a multipole of high electric moment capable of exerting an attraction on other bi- or multipoles over a certain sphere of influence, thus tending to the formation of aggregates. The large volume of the protein molecules implies that the multipoles are unusually close to one another, and the orientation of the molecules is equivalent to a 3–dimensional net-structure. Sackur

found that alkali increased the viscosity of solutions of sodium caseinate and he attributed the viscosity to the caseinate ions, an assumption which accords with Robertson's thesis. Hardy also stated that protein ions confer viscous character to protein solutions and Bottazzi and Loeb find viscosity is a minimum at the isoelectric point. Now, on Robertson's theory, when alkali is added to casein, what happens is as follows : the combination of successive equivalents of alkali causes a pair of bonds between diketopiperazine rings to open out and another pair of ions forms.[1]

Hitchcock [2] has adversely criticised the above theory that a protein chloride would not yield chloride ion on dissociation, as he finds several protein chlorides to undergo ionisation similar to that of ammonium chloride.[3] Sandstrom,[4] too, points out several weaknesses in Robertson's theory and enphasises that none of the data supporting the theory of chemical binding extend on the acid side beyond pH 2·5—3·0, and on the alkaline side beyond pH 11·0.

LOEB'S THEORY OF PROTEIN BEHAVIOUR

A mass of experimental work concerning the amphoteric reaction of proteins, and their behaviour particularly at the isoelectric point, has been carried out by Loeb,[5] and his important observations must now be briefly outlined.

Gelatin has an isoelectric point at pH 4·7. Combination with acid occurs at pH $< 4{\cdot}7$ and with alkali at pH $> 4{\cdot}7$. Experimental proof was given that when a salt, *e.g.*, $NiCl_2$, is added to gelatin solutions of varying pH values, nickel gelatinate is only possible when the pH exceeds 4·7. Similarly, if $K_4Fe(CN)_6$ be the salt added, gelatin $-Fe(CN)_6$ is formed, only if the pH is less than 4·7.

Loeb adduced evidence that proteins combine with acids and bases stoichiometrically.[6] Thus, the number of cubic centimetres of 0·1 N alkalis (KOH, NaOH, $Ca(OH)_2$, $Ba(OH)_2$) in 100 c.c. solution necessary to bring a 1 per cent. solution of isoelectric protein to the same pH value, when plotted against the pH value of the protein solution, all fall on one graph, *i.e.*, exhibit the course of true stoichiometric combination. That proteins combine chemically with HCl was demonstrated [7] by measuring the concentration

[1] Bacon, *J. Phys. Chem.*, **33,** 1843 (1929), supports Robertson's views on viscosity in HCl-gelatin systems. Cf. Frankel, *Kolloid-Z.*, **45,** 355 (1928).

[2] *J. Gen. Physiol.*, **5,** 383 (1923).

[3] A view held by Pauli.

[4] *J. Phys. Chem.*, **34,** 1073 (1930).

[5] His work is summarised in his book, " Proteins and the Theory of Colloidal Behaviour " (New York, 1922).

[6] Supported by Bacon and Ferguson, *J. Am. Chem. Soc.*, **49,** 1938 (1927) ; cf. Rawlins and Schmidt, *J. Biol. Chem.*, **82,** 709 (1929), on the stoichiometric combination of acid dyes with proteins. *Vide* also, Chapman, Greenberg and Schmidt, *ibid.*, **72,** 707 (1927), and the discussion of this paper by Gortner, Hoffman and Sinclair, *Am. Coll. Symp. Monog.*, **5,** 196 (1928).

[7] *Science*, **56,** 731 (1922).

of chloride ions in the protein-chloride solutions. As example, varying amounts of 0·1 N. HCl were present in 100 c.c. of 1 per cent. solutions of originally isoelectric gelatin and the pH and pCl values determined with the hydrogen and the calomel electrode respectively. Whilst the pCl was unchanged, the pH was higher, *i.e.*, part of the hydrogen united with NH_2 and NH groups of the protein molecule, whereas the chlorine remained free.

Loeb pointed out that protein salts are strongly hydrolysed. Considering the addition of HCl to isoelectric protein, there is partial combination of the acid with the protein, so that in solution equilibrium attains between :—

free HCl —— isoelectric protein ——·protein chloride.

Sufficient acid finally converts all the isoelectric protein to protein chloride.

Two statements [1] of Loeb's are of importance to the student owing to the many criticisms of this work by other prominent investigators :—

(1) " Proteins react with acids and alkalies in the same way as do amphoteric crystalloids like amino acids. If the methods for measuring the hydrogen ion concentrations of protein solutions had been employed by the colloid chemists *nobody would have thought of suggesting that proteins react with acids and alkalies according to the empirical adsorption formula* of Freundlich instead of stoichiometrically."

(2) " The colloidal behaviour of proteins shows itself in a peculiar effect of electrolytes—acids, alkalies or salts—on such properties as the swelling of gels or the osmotic pressure or viscosity of protein solutions. All these properties, swelling, osmotic pressure, viscosity, are affected by electrolytes in a very similar way ; suggesting that all are due to the same cause. We shall see that by giving the explanation for one of these properties, osmotic pressure, we shall by implication give the explanation for all of them."

Osmotic pressure determinations of protein solutions such as gelatin, crystalline egg albumin, casein and edestin were made by Loeb, using 1 gr. dry weight of originally isoelectric protein in 100 c.c. solution, which solution also included various amounts of 0·1 N. acid. The solutions were contained in collodion bags and suspended in protein-free water, which was initially at the same pH value as the solution inside (using the same acid). After 18 hours, osmotic pressure measurements were made. Fig. 6 is reproduced from Loeb's publication [2] and shows that the osmotic pressure varies with the pH value and with the valency of the anion of the acid present. The osmotic pressure is a minimum at the isoelectric

[1] The italics are by the present writer.
[2] *Science*, **56,** 731 (1922).

point and reaches a maximum at a higher acidity. The nature of the acid does not influence the osmotic pressure of protein solutions, but the valency does : " All acids which are monobasic on the acid side of the isoelectric point of a protein influence its osmotic pressure in the same way, and this influence is considerably greater than the influence of strong dibasic acids like H_2SO_4."

When alkali was added to isoelectric protein, a maximum osmotic pressure was again observed, all alkalies with monobasic cations (Li, K, Na, NH_4) having the same effect at the same pH value. Alkalies with dibasic cations (Ca, Ba) act similarly, the graph now being about one-half as high as with monovalent cations. According to Loeb, the graphs connecting the influence of acids and salts on osmotic pressure are practically identical to those connecting the influence of the same electrolytes on swelling and viscosity.

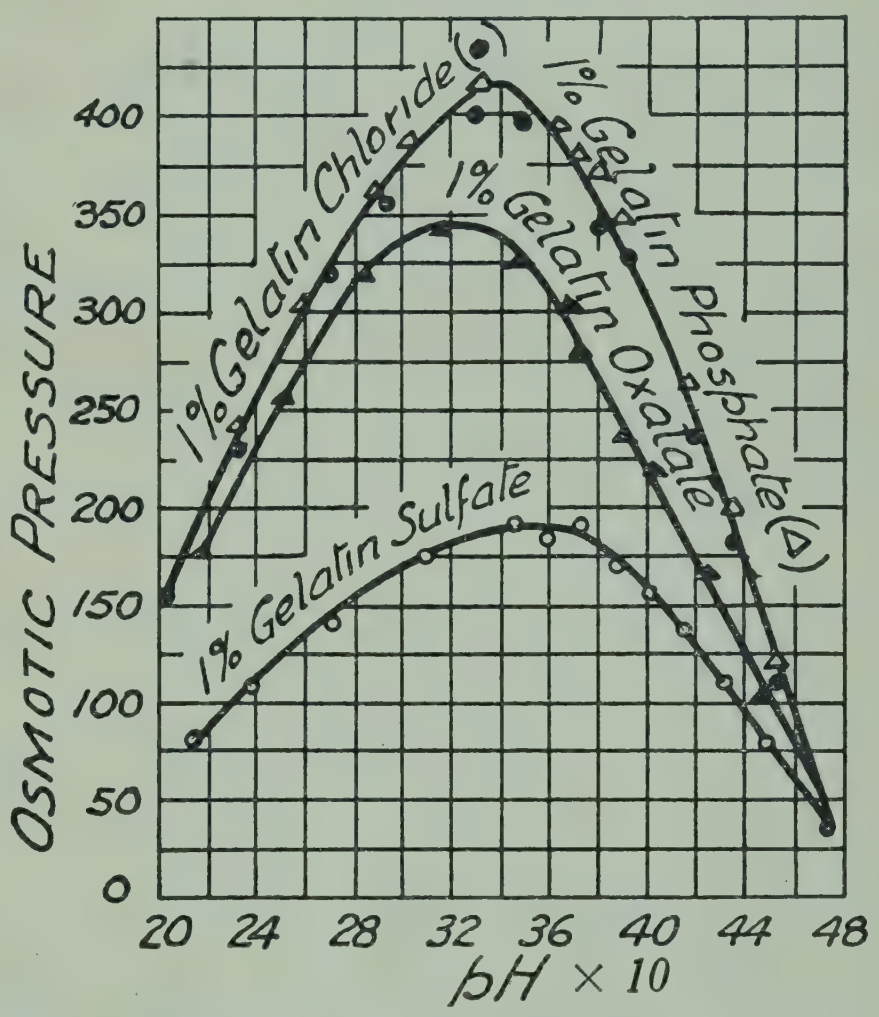


FIG. 6.—The osmotic pressure of protein solutions in relation to the pH value and the valency of the anion of the acid used.

To account for his results, Loeb argued that when acid or alkali was added to a solution of isoelectric protein, an ionisable protein salt resulted, in amount dependent on the quantity of added acid or alkali. This ionisation [1] of the protein is the cause of the colloidal behaviour, due to the inability of protein ions to diffuse through membranes, which are readily permeable to crystalloidal ions. Such membranes are collodion, parchment, the walls of capillaries and probably of all cells. In such a case, there arises an unequal distribution of the diffusible crystalloidal ions on the opposite sides of the membranes, and herein lies the origin of the colloidal behaviour of proteins. This phenomenon of ionic redistribution is dealt with by Donnan [2] in his theory of membrane equilibria, of which the following are the main outlines.

[1] " Proteins and the Theory of Colloidal Behaviour " (1922), p. 42 ; Hitchcock, *J. Gen. Physiol.*, **5,** 383 (1923) ; Manabe and Matula, *Biochem. Z.*, **53,** 369 (1913).

[2] *Z. Elektrochem.*, **17,** 572 (1911) ; *Chem. Reviews*, **1,** 73 (1924) from which the present account is mainly taken.

DONNAN'S THEORY OF MEMBRANE EQUILIBRIA

If a membrane separates two solutions containing electrolytes, and this membrane is permeable to the majority of the ions, but impermeable to at least one of them, certain peculiar physico-chemical effects are apparent, as Wilhelm Ostwald first pointed out. The theory of the phenomena involved was worked out by Donnan, and owing to the remarkable use of Donnan's theory by Loeb, and by Procter and Wilson, it has assumed considerable importance in the literature on the colloid chemistry of the proteins.

At the onset it should be realised that Donnan has pointed out that this theory rests simply on two assumptions :—

(1) The existence of equilibrium.

(2) The existence of certain constraints which restrict the diffusion of one or more electrically charged or ionised constituents.

Thus Donnan emphasises the very important fact that the equations deduced from his theory are just as valid whether one considers —

(*a*) "colloidal units," which ionise or carry an electric charge because of adsorbed ions, or

(*b*) simple molecules which undergo ionisation by the loss or gain of electrons.

It is only necessary for the theory that there should be the same constraint exercised on the simple molecules or the ionic micelles, namely, inability freely to diffuse through the membrane. Such constraint prevents the equal distribution of otherwise freely diffusible ions on both sides of the membrane, and therein lies the origin of the concentration, osmotic and electrical effects explained on Donnan's theory. Illustrations of constraints imposed on ionic diffusion are : (*a*) adsorption of ions on a solid wall ; (*b*) in a gelatin hydrochloride gel, the gelatin ions are restrained from diffusion by the cohesion of the structural network and the free diffusion of an equivalent amount of anions of the acid is likewise restricted.

ILLUSTRATIONS OF MEMBRANE EQUILIBRIA

Consider a semi-permeable membrane separating two solutions of electrolytes possessing a common ion. Thus, consider NaCl and NaR, where $Na^{\cdot}$ and Cl' are freely diffusible through the membrane, whilst R' is not. Then on one side of the membrane are the ions $Na^{\cdot}$, Cl' and R', whilst on the other side are the ions $Na^{\cdot}$ and Cl'. It can now be shown on thermo-dynamical grounds that when the system is in equilibrium *the products of the concentrations of a pair of diffusible anions and cations are equal on the two sides of the membrane.* That is

$$[Na^{\cdot}]_2\,[Cl']_2 = [Na^{\cdot}]_1\,[Cl']_1$$

where 1 and 2 stand for the respective solutions on either side of the membrane.

Making the assumptions that : (*a*) the volume of the liquid is the same on either side of the membrane, (*b*) there is complete ionisation of the electrolytes, we find that for different *initial* concentrations of NaCl and NaR the *equilibrium* concentrations are as shown in Table 9.

TABLE 9

Initial Concentration of NaR in Solution 1.	Initial Concentration of NaCl in Solution 2.	NaCl Transferred from Solution 2 to 1 (per cent.).	Distribution Relation of Total NaCl. $\frac{[NaCl]_2}{[NaCl]_1}$
0·01	1·0	49·7	1·01
0·1	1·0	47·6	1·1
1·0	1·0	33·0	2·0
1·0	0·1	8·3	11·0
1·0	0·01	1·0	99·0

The important fact deserving emphasis is that although the membrane permits ready permeability to NaCl, the presence of NaR with its non-permeable anion R′, can effectively inhibit the permeability of NaCl in one direction ; indeed, sufficient NaR can practically entirely render sodium chloride diffusion unidirectional.

Another interesting case concerns the system which does not possess a common ion, *e.g.*, when solution 1 contains NaR and solution 2 contains KCl. On one side of the membrane we have initially the ions $Na^{\cdot}$ and R′ (non-permeable), and on the other side the ions $K^{\cdot}$ and Cl′. In the equilibrium state of the system, the ions in solution 1 will be $Na^{\cdot}$, $K^{\cdot}$, Cl′ and R′, whilst in solution 2 they will be $Na^{\cdot}$, $K^{\cdot}$, and Cl′. Ionic equilibrum is established when :

$$\frac{[Na^{\cdot}]_1}{[Na^{\cdot}]_2} = \frac{[K^{\cdot}]_1}{[K^{\cdot}]_2} = \frac{[Cl']_1}{[Cl']_2}.$$

A sufficient concentration of NaR will check the permeability of the membrane to Cl′, whilst allowing permeability to $K^{\cdot}$.

Reverting to the first system considered (NaCl and NaR), Donnan's theory enables the calculation of the potential difference [1] arising at the membrane when the ionic distribution reaches the equilibrium state. The potential difference is

$$E = 0{\cdot}058 \log \frac{[Na^{\cdot}]_2}{[Na^{\cdot}]^1} = 0{\cdot}058 \log \left(1 + \frac{[NaR]_0}{[NaCl]_2}\right)$$

where $[NaR]_0$ is the initial concentration of NaR in solution 1 and $[NaCl]_2$ the concentration of NaCl in solution 2. For a change in

[1] For a full discussion see Michaelis in Alexander's " Colloid Chemistry," Vol. I. (New York, 1926), pp. 499 *et seq.* Cf. also Oakley, *J. Phys. Chem.*, **30,** 902 (1926).

the ratio $[NaR]_0/[NaCl]_2$ from 1 to 100, the value of E changes from + 0·017 to + 0·116. Thus, the larger the value of $[NaR]_0$ compared to $[NaCl]_2$, the greater is the difference of potential set up. In other words, the concentration of the electrolyte with the non-permeable ion must be large compared to the concentration of the salt with two permeable ions on the opposite side of the membrane.

THE PROCTER-WILSON THEORY

An important application of the Donnan theory of membrane equilibria has been made by Procter and Wilson [1] in considering the effect of acids and salts on the swelling of gelatin. Loeb has attached the highest importance to this discussion. Simply stated the theory is that the immersion of a block of gelatin gel in a solution of HCl causes the solution to fill up the molecular interstices of the gel structure. Gelatin chloride forms and ionises, but whereas the Cl ions exist in the interstitial solution, the corresponding gelatin cations are not in solution in the strict sense, since they form part of the gel network. The Cl ions are balanced only by the positive electrical charges on the network of gelatin and, in consequence, there is an unequal distribution of all ions between the interstitial solution and the main (external) solution, the total concentration of ions in the gel being always greater than in the main solution. The anions of the gelatin chloride tend to diffuse into the main solution and so exert an attraction on the cations which form part of the gel structure. This causes the volume of the gel to increase in direct proportion to the excess of concentration of diffusible ions of the gel over that of the main solution. The greater the proportion of the gelatin existing in the ionic (*i.e.*, electrically charged) form the greater is the extent of the swelling produced. Counteracting such swelling is the presence of any ions which do not increase the proportion of ionised gelatin.

In applying the Donnan reasoning, the absence of a membrane is immaterial, since the inability of the gelatin ions to leave the gel network imposes the necessary condition of restraint.

Let the protein G form an elastic gel which is insoluble in water, but readily permeable to water and to ions in solution. The gel is assumed to form chemical union with $H^{\cdot}$, but not the anion A', of the acid HA, *i.e.*, the compound GHA ionises to the ions $GH^{\cdot}$ and A'.

In an aqueous solution of HA let us immerse a strip of the gel equal to 1 millimol of G. Diffusion of the acid into the gel leads to removal of hydrogen ions from solution owing to their union with the protein and the absorbed solution becomes more concentrated

[1] Summarised by Wilson in (*a*) *Brit. Assoc. Colloid Repts.*, **3,** 51 (1920); (*b*) *Am. Coll. Symp. Monog.*, **1,** 210 (1923); (*c*) "The Chemistry of Leather Manufacture," Vol. I, 129 (1928).

in respect to A′ than to H˙. The union of each hydrogen ion with the protein adds one positive electrical charge to the insoluble gel which is balanced by one anion (A′) in the absorbed solution. Now in the main solution the concentration of H˙ and A′ must be equal, but in the absorbed solution the anionic concentration is greater than the hydrogen ion concentration.

When the distribution of ions in the absorbed and the main solutions reaches equilibrium :—

$[H^{\cdot}] = [A'] = x$ . . in the main solution.
$[H^{\cdot}] = y$, and $[A'] = z$. in the gel phase.

Corresponding to y will be [A′], the concentration of free anions of the acid HA in the gel phase ; the total value of [A′] in the gel phase in thus $(y + z)$, z being the concentration of anions united to the gelatin cation. Applying Donnan's thermodynamic reasoning, the following equation is reached :—

$$x^2 = y\,(y + z).$$

Thus, *equilibrium attains when the product* [H˙] [A′] *has the same value in the main solution and in the gel phase.*

From the above equation it is evident that

$$2y + z > 2x,$$

i.e., the sum of the concentrations of diffusible ions in the gel phase exceeds the sum of the concentrations of these ions in the main solution. This concentration difference is the cause of the excess osmotic pressure of the gel ; water enters the gel and leads to swelling.

It should be emphasised that at equilibrium $x > y$, *i.e.*, the acidity of the main solution is greater than that of the gel phase. Again, as Wilson remarks, " all monobasic acids should produce the same degree of swelling of gelatin for any fixed hydrogen ion concentration, under constant conditions, provided the gelatin salts formed are ionised to the same extent."

The theory [1] is easily extended to account for the repression of swelling by neutral salts and to explain the alkali-protein equilibrium, where similar protein behaviour is predicted, provided no chemical change occurs beyond that of salt formation. The valency effect is similar in both acid and alkali solutions.[2]

LOEB'S APPLICATION OF THE PROCTER-WILSON THEORY

In an extensive series of papers, Loeb adopted the Procter-Wilson theory of the swelling of gelatin gels and believed he furnished

[1] See also the theory of Tolman and Stearn, *J. Am. Chem. Soc.*, **40,** 264 (1918), and its extensions by Tolman and Bracewell, *ibid.*, **41,** 1503, 1511 (1919).

[2] See the criticism by Neville and Theis, *Am. Coll. Symp. Monog.*, **7,** 42 (1930).

support for it. For instance, he showed that the degree of swelling by polybasic acids, which combine as such with gelatin, is much less than that caused by monobasic acids, since there are fewer anions associated with an equivalent weight of gelatin. Loeb maintained the thesis that "two laws of classical chemistry suffice to explain the colloidal behaviour of proteins quantitatively and mathematically, and these two laws are the stoichiometrical law and Donnan's theory of membrane equilibria."

The valency of an ion and not its nature is at the basis of Loeb's theory of the colloidal behaviour of proteins, and this led him closely to examine the influence of series of ions on various physico-chemical properties of proteins in solution. He was thus led to contradict the existence of the long-recognised Hofmeister ion series.

THE HOFMEISTER ION SERIES

Hofmeister [1] found that the effects of various anions on certain physico-chemical properties of proteins such as swelling, were specific, and that a series of anions could be arranged according to their efficiency, this order bearing no relation to the nature of the cation. Thus, the action of salts in producing turbidity in a solution of egg-white (neutral or weakly alkaline) led to the following series in order of efficiency :—

$$\text{citrate} > \text{tartrate} > SO_4 > \text{acetate} > Cl > NO_3 > ClO_3.$$

Such ionic series have been termed by Freundlich [2] *lyotropic series* and he suggests a correspondence with the order of hydration of the ions.

Cationic effects are also possible, *e.g.*, Rettig [3] measured the electrical conductivity of gelatin solution with and without added salts. The depression of conductivity by salts of the same cation gave the efficiency order of cations : $SO_4 > Cl > Br$.

Although numerous investigators have reported Hofmeister ion series in various connections, Loeb argued that "the Hofmeister ion series are only the results of a methodical error," this being "the failure of the former authors to measure the hydrogen ion concentration of their protein solutions or gels and to compare the effect of acids at the same pH of the protein solution or protein gel." Loeb's experiments showed that [4] : "When acids are added to a solution or a gel of isoelectric protein, the osmotic pressure, and, in

[1] *Arch. exptl. Path. Pharmakol.*, **24,** 247 (1888) ; **25,** 1 (1888—89) ; **27,** 395 (1890) ; **28,** 210 (1891).

[2] "Colloid and Capillary Chemistry" (London, 1926), p. 58. See Michaelis, "The Effect of Ions in Colloidal Systems" (Baltimore, 1925), p. 83.

[3] *Kolloid-Z.*, **27,** 165 (1920). See also the work of Kondo *et al*, on rice glutenin for anionic effects (K. salts) and cationic effects (chlorides), *Mem. Coll. Agr. Imp. Univ.*, No. **5,** 1—47 (1928).

[4] "Proteins and the Theory of Colloidal Behaviour," 2nd Edn. (1924), p. 131, italics by the present writer.

the case of some proteins, the viscosity of the protein solution, and the swelling of gels are affected in a similar way. The values of these three properties rise until a maximum is reached, which is followed by a drop if more acid is added. All the acids with monovalent anion act quantitatively alike *when their effect is compared at the same* pH of the protein solution or the protein gel ; and all the acids with bivalent anion act alike, but the effect of the latter acids is considerably lower than that of the former. It follows therefore, that *only the valency but not the chemical nature of the anion* of the acid has an effect on these three properties of proteins. *This contradicts the so-called Hofmeister anion series.*"

That Loeb greatly exaggerated the significance of the hydrogen ion concentration (important though it undoubtedly is) has been the opinion of such prominent workers as Kruyt,[1] McBain,[2] Gortner [3] and others.[4]

GORTNER'S INVESTIGATIONS

A striking demonstration of the existence of a lyotropic series as affecting protein systems has been given by Gortner, Hoffman and Sinclair.[5] Wheat flour was used as the source of proteins on which was studied the peptising action of aqueous solutions of twenty-one inorganic salts, mostly varying in concentration from 0·5 N. to 2·0 N. (see p. 148).

There appeared a very pronounced lyotropic series effect, particularly evident with K halides, the order here being KF<KCl<KBr<KI. The importance of maintaining all the solutions and extracts at essentially identical pH values was realised, thus excluding any influence due to the concentration of hydrogen ions. Again, such salts do not undergo hydrolysis in aqueous solution. The total protein extracted varied from 13·07 per cent. (KF) to 68·39 per cent. (KI), due solely to a specific anion effect, the salts in this particular example all being at 1 N. concentration. Additional conclusions reached by the authors were :—

(*a*) There is an evident Hofmeister series of anions, the peptising action increasing in the order $F<SO_4<Cl<$tartrate$<Br<I$.

(*b*) There is less evident, yet distinct, Hofmeister series of cations, the peptising action increasing : Na<K<Li<Ba<Sr<Mg<Ca.

[1] " Colloids " (New York, 1930), p. 249 ; Kruyt and Tendeloo, *J. Phys. Chem.*, **29,** 1303 (1925).

[2] *Am. Coll. Symp. Monog.*, **4,** 15 (1926).

[3] *Ibid.*, **5,** 194 (1928).

[4] Freundlich, " Colloid and Capillary Chemistry " (London, 1926), p. 837 ; Alexander, *Chem. Met. Eng.*, **27,** 368 (1922) ; Michaelis, Alexander's " Colloid Chemistry," Vol. I., 506 (New York, 1926), p. 506 ; Pauli, *ibid.*, Vol. 2 (New York, 1928), p. 229 ; Bayliss, " Interfacial Forces and Phenomena in Physiology " (London, 1923), pp. 67 and 95 ; Ferguson, *Am. Coll. Symp. Monog.*, **5,** 177 (1928) ; Fanselow, *ibid.*, **6,** 237 (1928).

[5] *Am. Coll. Symp. Monog.*, **5,** 179 (1928). See the criticism of this by Saunders, *J. Am. Chem. Soc.*, **53,** 696 (1931).

(*c*) "The specific properties of certain anions or cations may be so great as to entirely overshadow relatively large changes in hydrogen-ion concentration."

This investigation certainly reacts most unfavourably towards Loeb's theory, but it is now only one of many publications pointing to the limitations of the theory.[1]

CRITICISMS OF LOEB'S THEORY

Gortner [2] has pointed out that as proteins are amphoteric, they will react with acids and bases, but, at the same time, since the proteins are lyophilic colloids, adsorption of hydrogen and hydroxyl ions can occur at their surfaces, possibly to an extent under some conditions that the colloidal behaviour will outweigh the stoichiometry of primary valencies. The setting up of a Donnan equilibrium is no proof of stoichiometrical reaction, as an adsorption complex might also furnish ions, diffusible and non-diffusible.

Kopaczewski [3] has made detailed criticisms of Loeb's technique and conclusions, and considers that Loeb's data rather favour the existence of adsorption compounds. This is also the view of Rinde,[4] who used Loeb's data on the addition of HCl to gelatin (isoelectric) and followed the osmotic pressure and P.D. changes. Applying Langmuir's adsorption theory of residual valencies, Rinde obtained surprising agreement between the theoretical amounts of adsorbed ions and the calculated amounts based on determinations of the activities inside and outside the membrane. Most probably the "reaction" between gelatin and HCl is an adsorption process, the hydrogen ions being bound to the gelatin by the residual valencies of the latter. Loeb's data also permit the calculation of the adsorption of neutral HCl molecules by the gelatin. Rinde points out that in the isoelectric zone real chemical reaction may occur.

Other criticisms of Loeb's reasoning are given by Kruyt,[5] Hill,[6] Bancroft,[7] Johlin [8] and others. Bancroft points out that Loeb's salt solutions were so dilute as to preclude his detecting any adsorption of the cation or anion of the salt when working with acid or alkali solutions. Again, Bancroft pertinently remarks: "Loeb's conclusions as to the existence of definite compounds

[1] See for instance, Ostwald, Kuhn and Bohme, *Kolloidchem. Beihefte*, **20,** 412 (1925); Shukoff and Stschoukareff, *J. Phys. Chem.*, **29,** 285 (1925).

[2] "Outlines of Biochemistry" (New York, 1929), pp. 285 and 412.

[3] "L'Etat Colloidal et l'Industrie," Vol. I., 40 *et seq.* (Paris, 1925); Alexander's "Colloid Chemistry," Vol. I., 547 *et seq.* (New York, 1926).

[4] *Phil. Mag.* (7), **1,** 32 (1926).

[5] *Am. Coll. Symp. Monog.*, **5,** 17 (1928).

[6] *Proc. Roy. Soc.*, **A 102,** 705 (1923). Cf. Hitchcock, *J. Gen. Physiol.*, **5,** 661 (1922—23), and Hill, *ibid.*, **6,** 91 (1923).

[7] "Applied Colloid Chemistry" (New York, 1926), p. 337; *J. Phys. Chem.*, **34,** 449 (1930).

[8] *J. Biol. Chem.*, **86,** 231 (1930).

depend on the assumption that he is dealing with true solutions and will fall with that assumption."

RECENT INVESTIGATIONS ON THE PROTEINS

Recent work is serving to clear up the discrepancies between Loeb's conclusions and the conflicting findings of other investigators, and supports fully the conception of proteins as typical colloids able to form adsorption compounds.

An extensive investigation by Hoffman and Gortner [1] dealt with the reactions of numerous proteins with acids and bases. The authors concluded that two types of combination are possible, depending upon the pH range, in which the reaction takes place :—

Type I. is chemical and occurs in the pH range 2·5—10·5.

Type II. is an adsorption-combination and occurs when the pH is $< 2{\cdot}5$ and $> 10{\cdot}5$.

Regarding these types, when the log (amount of acid or alkali bound) is plotted against log (original acid or alkali concentration) or against log (final pH), type II. leads to straight line graphs (see p. 319), *i.e.*, evidence of adsorption. Again, at the higher concentrations of acid and alkali (type II.) all the proteins used, irrespective of chemical composition, bind approximately the same amount of acid or alkali at equal concentrations of protein.[2] The adsorption-combination is also supported by the fact of there being a negative temperature coefficient of the acid or alkali binding at the higher concentrations of hydrogen and hydroxyl ions.

In criticising this investigation, Stearn [3] points out regarding type II. that the conclusions drawn from the temperature coefficient take no account of the possible effect of temperature on the difference in activity of hydrogen ion in pure water and in the protein solutions. Stearn's own results on the acid- and base-binding of gelatin lead him to conclude " that the binding due to adsorption in the case of gelatin is not significant compared to that due to chemical neutralisation." There will always be some adsorption-binding in the case of proteins but, in general, such adsorption-binding of acid and alkali cannot be referred to the great majority of the total amounts bound.

Bancroft,[4] whilst agreeing that Gortner's work is of much importance, pointed out that the results are not convincing, as Gortner worked with too dilute protein solutions, assumed the absence of protein error and ignored hydrolysis.

Extensive phase rule studies on the proteins are now being

[1] *Am. Coll. Symp. Monog.*, **2,** 209 (1925). Supported by Sandstrom, *J. Phys. Chem.*, **34,** 1097 (1930) ; *vide* Fanselow, *Am. Coll. Symp. Monog.*, **6,** 237 (1928).

[2] This assumes considerable significance in the light of Svedberg's recent work (see p. 31).

[3] *J. Gen. Physiol.*, **11,** 377 (1928).

[4] *J. Phys. Chem.*, **29,** 1482 (1925).

submitted from Bancroft's laboratory and results of the first importance have been published.[1]

Adsorption of acid, base or dye by silk, wool, *etc.* is readily determined by treating the fibres with solutions of varying concentration and inspection of the graph obtained by plotting the amount taken up against the amount taken up against the amount residual in solution, enables differentiation between adsorption (= smooth curve) or the coexistence of two solid phases (= curve with a flat in it). With proteins in solution, which are peptised, one cannot accurately analyse the two or more phases. Bancroft, therefore, eliminates the solution phase, treating the powdered protein with gaseous NH_3 or HCl, noting the amounts of gas taken up and the corresponding pressures. The formation, for example, of ammonium proteinate shows a constant gas pressure until all the protein is converted into the ammonium compound. Adsorption of ammonia would lead to a smooth curve characteristic of adsorption phenomena. Adding moderate amounts of water does not influence the type of the curve, although it displaces the curve.

In this way, it was found that "casein and gelatin adsorb ammonia readily with no evidence of the formation of any definite compound." Further investigation permitted the same conclusion in the case of ammonia and zein, arachin, fibrin and gliadin. On the other hand, definite compounds are formed with HCl by casein, arachin, fibrin, gliadin, and edestin, but not zein.

THE ISOELECTRIC POINT

The electric charge carried by suspended colloid particles can be readily demonstrated by studying their motion under the influence of an electric field. The particles migrate (cataphoresis) to the electrode of opposite sign to that of the charge on the particles. The direction of movement of the particles can be reversed by suitable addition of acid or alkali, as the case may be, depending on the original sign of the electric charge. At a certain concentration of hydrogen ions, therefore, there will be an indifferent point, the colloid particles showing no cataphoretic movement, *i.e.*, the potential difference between the particles and the medium is zero. Hardy[2] termed this the isoelectric point.

In the investigation of proteins the isoelectric point assumes paramount importance. In solution a protein is amphoteric, but although dissociation can furnish $H^{\cdot}$ and OH' ions, acidic or basic characteristics predominate, usually the former. At the isoelectric point we have the conception (due to Michaelis)[3] that the concen-

[1] Bancroft, Barnett and Belden, *Am. Coll. Symp. Monog.*, **7,** 151 (1930); Bancroft and Barnett, *J. Phys. Chem.*, **34,** 449, 753, 1217, 1930 (1930).

[2] *Proc. Roy. Soc.*, **68,** 110 (1910)

[3] "Handbuch der Biochemie" (Jena, 1913), Supplement, p. 24; cf. Sørensen, *Ergebnisse Physiol.*, **12,** 503 (1912); Michaelis, "Die Wasserstoffionkonzentration"

tration of protein anions is the same as that of the protein cations. Loeb's [1] conception is: "the isoelectric point of a protein is that hydrogen ion concentration in which the protein exists practically in a non-ionogenic (or non-ionised) condition, being able to form practically neither metal proteinate nor protein-acid salt."

Following Michaelis,[2] consider a protein which can furnish anions (A^-) and cations (C^+), x being the concentration of undissociated protein. Let the acid dissociation constant and the basic dissociation constant be k_a and k_b respectively. For equilibrium,

$$k_a = \frac{[A]\,[H]}{x} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$k_b = \frac{[C]\,[OH]}{x} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

At the isoelectric point, [A] = [C], so that,

$$\frac{k_a}{k_b} = \frac{[H]}{[OH]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Since k_w, the dissociation constant of water, equals [H] [OH],

$$[H] = \sqrt{\frac{k_a}{k_b} . k_w} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Thus Michaelis' isoelectric point is fixed by equation (4). His interesting theory cannot be tested since the dissociation constants involved are unknown, and, as Svedberg [3] points out, it is still conjecture whether the Michaelis isoelectric point coincides with the cataphoretic isoelectric point. On the Michaelis theory the concentration of undissociated protein can be shown to be a maximum at the isoelectric point and this agrees with experimental findings that proteins have a minimal solubility at their isoelectric points, since the undissociated protein is always less soluble than the dissociated. A limitation of the theory [4] is the non-recognition of the part played in cataphoresis by ions other than $H^{\cdot}$ and OH'.

Recently, Harris [5] has put forward evidence to support the theory that at the isoelectric point the protein molecule is not a practically non-ionised molecule, but exhibits pronounced ionisation as both acid and base. He calculates, for example, that the ionisation of

(Berlin, 1922), p. 52. An extension of the theory is given by Kuhn, *Z. physik. Chem.*, **114,** 44 (1924).

[1] "Proteins and the Theory of Colloidal Behaviour" (New York, 1924), p. 10.

[2] Important references in this connection are: Michaelis and co-workers, *Biochem. Z.*, **24,** 79 (1910); **27,** 38 (1910); **28,** 193 (1910); **30,** 143 (1910); **33,** 456 (1911); **47,** 250 (1912); Sørensen, *Z. physiol. Chem.*, **103,** 192 (1918), and *J. Gen. Physiol.*, **8,** 568 (1927); Eckweiler *et al.*, *ibid.*, **3,** 305 (1921), criticised by Michaelis in "Hydrogen Ion Concentration" (London, 1926), p. 66; Cohn, *J. Gen. Physiol.*, **4,** 703 (1922); Stearn, *ibid.*, **10,** 315 (1926).

[3] "Colloid Chemistry" (New York 1928), p. 242.

[4] *Vide* Michaelis and Rona, *Biochem. Z.*, **94,** 225 (1919) and the important data by Küntzel, *ibid.*, **209,** 326 (1929).

[5] *Proc. Roy. Soc.*, **B 97,** 379 (1925).

isoelectric gelatin as a base is at least 34 per cent. The protein molecule being electrically neutral is ionised equally as an acid and as a base and, therefore, the ionisation is very marked.

Equation (4) indicates that the isoelectric point of an ampholyte is independent of concentration.[1] Temperature also has but slight influence.[2]

Some typical isoelectric points [3] are collected in Table 10.

TABLE 10. ISOELECTRIC POINTS OF TYPICAL PROTEINS

Protein.	Isoelectric Point (pH).	Observer.
Albumin (egg)	4·55	Tiselius.[4]
	4·8	Sørensen ; [5] Michaelis and Nakashima.[6]
	4·75	Hartl and Starlinger.[7]
	5·5	Ettisch *et al.*[8]
Albumin (milk)	5·2	Sjögren and Svedberg.[9]
	4·55	Csonka *et al.*[10]
Albumin (wheat bran)	4·2	Csonka *et al.*[10]
Casein	4·85	Csonka *et al.*[10]
	4·74	Rona and Michaelis.[11]
Fibrinogen	4·81	Hartl and Starlinger[7].
Gelatin	4·7	Michaelis and Grineff.[12]
Gliadin (wheat)	7·10	Haugaard and Johnson.[13]
	6·45	Csonka *et al.*[10]
	6·5	Tague.[14]
Globin	7·5	Roche.[15]
Globulin (serum)	5·5	Svedberg.[16]
Globulin (wheat bran)	5·5	Csonka *et al.*[10]
	5·14	Hartl and Starlinger.[7]
Glutenin (wheat)	7·0	Tague.[14]
	5·15—5·39	Konda *et al.*[18]
Hæmocyanin (*Helix*)	5·05	Tiselius.[4]
Hæmoglobin	6·8	Roche.[15]
Peptone	5·54	Mori.[17]
Zein (corn)	6·2	Csonka *et al.*[10]

[1] But see Eckweiler *et al*, *J. Gen. Physiol.*, **3,** 307 (1921).
[2] Zhukov and Matusevich, *J. Russ. Phys. Chem. Soc.*, **61,** 1683 (1929).
[3] Many values are given by Hoffman and Gortner, *Am. Coll. Symp. Monog.*, **2,** 354 (1925).
[4] Quoted by Svedberg, *Trans. Faraday Soc.*, **26,** 737 (1930).
[5] *Compt. rend. trav. lab. Carlsberg*, **12,** 163 (1915–17).
[6] *Biochem. Z.*, **143,** 484 (1923).
[7] *Z. ges. exptl. Med.*, **60,** 289, 315 (1928).
[8] *Naturwissenschaften*, **18,** 447 (1930).
[9] *J. Am. Chem. Soc.*, **52,** 3650 (1930).
[10] *Ibid.*, **48,** 765 (1926).
[11] *Biochem. Z.*, **28,** 193 (1910).
[12] *Ibid.*, **41,** 373 (1912).
[13] *Compt. rend. trav. lab. Carlsberg*, **18,** No. 2, 1—132 (1930).
[14] *J. Am. Chem. Soc.*, **47,** 418 (1925).
[15] *Compt. rend.*, **189,** 378 (1929).
[16] *Kolloid-Z.*, **51,** 23 (1930).
[17] *J. Biochem. Japan*, **7,** 411 (1927).
[18] *Mem. Coll. Agr. Kyoto Imp. Univ.*, No **5,** 1—47 (1928).

Determination of the Isoelectric Point. Various methods have been employed to determine the isoelectric point. Only brief reference can be made in the present volume, pointing out the theoretical principles involved.

(*a*) *Cataphoresis.* The isoelectric condition of the protein is determined by making cataphoresis observations in relation to change in the pH of the system. Except in the isoelectric zone, the protein migrates to the anode, if the system is at a higher pH value, and to the cathode, if at a lower pH value than the isoelectric pH zone.[1] Recent work shows, however, that the isoelectric point is not a function of the pH value alone ; it is a function also of the nature of other ions present in the system.[2] Adsorption of oppositely-charged ions, especially polyvalent ions, will modify the boundary electrical charge of the colloid as the work of Kruyt and Tendeloo [3] has shown in connection with the influence of $K_3Fe(CN)_6$ on the viscosity of a gelatin sol of pH value 4·4. In the case of proteins the special chemical relation with hydrogen ion has hitherto obscured the quite general action of anions and cations.

(*b*) *Coagulation with Alcohol.* Since the stability of proteins is at a minimum at the isoelectric point, coagulation with alcohol is a maximum.[4]

(*c*) *Viscosity.* This property has a minimum value at the isoelectric point.[5]

(*d*) *Turbidity.* Wintgen and Vogel [6] find maximum turbidity at the isoelectric point ; ultramicroscopic particles are responsible, but they gradually aggregate to form flocs.

(*e*) *Osmotic Pressure.* Hitchcock [7] has determined the isoelectric point of gelatin by observation of that pH value corresponding to minimum osmotic pressure.

(*f*) *Mastic Sol Method.* Michaelis and Davidsohn [8] had shown that when two proteins are mixed, the pH value of maximum precipitation depends on (1) the relative quantities of the proteins, and (2) their individual isoelectric points. By increasing the relative amount of one protein to a sufficiently large extent, the pH value of

[1] Experimental difficulties are discussed by Pauli, "Colloid Chemistry of the Proteins" (London, 1922), pp. 34 *seq.* See also, Cohn *et al.*, *J. Gen. Physiol.*, **2,** 145 (1919) ; Svedberg and Jette, *J. Am. Chem. Soc.*, **45,** 954 (1923) ; Abramson, *ibid.*, **50,** 390 (1928) ; Sheppard and Houck, *J. Phys. Chem.*, **34,** 2192 (1930) ; Landsteiner and Pauli, *Verhandl. Köng. inn. Med.*, **25,** 571 (1908).

[2] Svedberg, *Trans. Faraday Soc.*, **26,** 737 (1930) ; Freundlich, "Colloid and Capillary Chemistry" (London, 1926), p. 572 ; Kruyt "Colloids" (New York, 1930), p. 211.

[3] *J. Phys. Chem.*, **29,** 1303 (1925).

[4] Fenn, *J. Biol. Chem.*, **33,** 279, 439 ; **34,** 141, 415 (1918) ; Pauli, "Colloid Chemistry of the Proteins" (London, 1922), p. 38 ; Sheppard and Houck, *J. Phys. Chem.*, **34,** 2192 (1930).

[5] Cf. Pauli and Matula, *Kolloid-Z.*, **12,** 222 (1913).

[6] *Ibid.*, **30,** 45 (1922). *Vide* also, Kraemer, *Am. Coll. Symp. Monog.*, **4,** 104 (1926) ; Sheppard and Houck, *J. Phys. Chem.*, **34,** 2189 (1930).

[7] *J. Gen. Physiol.*, **12,** 495 (1929).

[8] *Biochem. Z.*, **30,** 144 (1910).

maximum precipitation practically coincides with the isoelectric point of this protein. In place of a second protein, a mastic sol can be used [1] prepared in buffer (acetate) solutions of varying pH values. Addition of gelatin, for example, causes flocculation at pH$<$4·7, but increasing the amount of gelatin brings the pH value of maximum flocculation to 4·7, a value unchanged by further addition of gelatin.

(*g*) *Precipitation.* A solution of the protein is added to a series of buffer solutions of various pH values; that pH value of the buffer corresponding to maximum precipitation is the isoelectric value.[2] A modification of the method due to Ssonka *et al.*[3] is to produce a solution of the protein by shaking an excess in buffers (pH4 — pH 6·8) directly, and effecting precipitation in the clear filtrate with tungstic acid. The pH value of the buffer filtrate in which was produced the least turbidity is the isoelectric value. The method cannot be used for very soluble proteins (gelatin, egg albumin) or for those whose solubility in the buffers is small (zein and edestin).

Tague [4] dissolved a constant amount of gliadin or glutenin (wheat) in a constant volume of mixtures of 0·01 M. solutions of primary and secondary sodium phosphates of known pH value and determined the change in pH value after shaking for eight hours. When the proteins were dissolved in a phosphate solution of pH$<$ the isoelectric point, the protein behaved as a base and increased the pH value. When the pH of the phosphate solution was greater than the isoelectric point, the protein functioned acidically, and the pH value was decreased. Curves were obtained by plotting as abscissæ, the pH value of the phosphate mixture and as ordinates the pH difference after solution of the protein. The point where no pH change is noticed is the isoelectric point, a value confirmed by solubility determinations.

Assuming the charge on a protein to be due to its dissociation as an amphoteric electrolyte, it follows that on the acid side, anions of any salt, and on the alkaline side, cations of any salt, will depress the dissociation of the protein through its common ion. Thus, Sørensen [5] determined the isoelectric point of egg-albumin by ascertaining that pH value which remains unchanged by the addition of a neutral salt.

(*h*) *Solubility.* The solubility of a protein is independent of the amount of it present only at the isoelectric point, *i.e.*, under other conditions, protein combines with acid or base and the concentration

[1] Michaelis and Nakashima, *ibid.*, **143,** 484 (1923).

[2] Michaelis and Davidsohn, *ibid.*, **30,** 144 (1910); Michaelis and Pechstein, *ibid.*, **47,** 260 (1912).

[3] *J. Am. Chem. Soc.*, **48,** 763 (1926).

[4] *Ibid.*, **47,** 418 (1925).

[5] *Compt. rend. trav. lab. Carlsberg,* **12,** 163 (1917); cf. Cohn, *Proc. Nat. Acad. Sci.*, **6,** 256 (1920) and *J. Gen. Physiol.*, **4,** 700 (1922).

of the protein compound is a function of the dilution. Cohn [1] has discussed the theoretical basis of this method, concluding that " the solubility of a protein at its isoelectric point is a fundamental physico-chemical constant, characterising and identifying the protein under investigation." The isoelectric points of serum globulin, tuberin and casein were thus determined.

MAXIMAL AND MINIMAL PROPERTIES

At the isoelectric point the characteristic properties of the proteins exhibit maxima and minima values. At a maximum are : analytical purity, turbidity, and surface tension lowering. At a minimum are : viscosity, optical rotation,[2] stability in solution, ash content, electrical conductance, osmotic pressure and membrane potential.

Regarding viscosity, it is considered [3] that the protein ions arising from acid or alkali union are more viscous than the undissociated protein, *i.e.*, they are more hydrated.

Considering the question of stability in solution, the difficult solubility of the undissociated protein reaches a maximum at the isoelectric point. The degree of hydration is of great importance, protein ions being heavily hydrated, but not the undissociated protein, and accordingly those phenomena connected with hydration are at a minimum. At the isoelectric point gelatin, glutin and natural albumin are not necessarily precipitated, whereas casein, salt-free globulin and acid albumin are. The former are hydrated in solution, the latter much less so. It follows readily that turbidity is a maximum at the isoelectric point, as is also precipitation by acetone or alcohol.[4] Pauli [5] holds the opinion that in the flocculation of a protein by alcohol, the removal of water from the protein is accompanied by an acid amide type of condensation of adjacent NH_2 and COOH groups.

Serum albumin and egg albumin were found by de Caro and Laporta [6] to have minimum surface tension values in solution at the isoelectric point. Bottazzi [7] has devoted much attention to the question of the surface tension lowering of water by proteins and has emphasised that not the ions, but the undissociated molecules [8] are responsible for the surface tension reduction and hence, at the

[1] *J. Gen. Physiol.*, **4,** 700 (1922).
[2] Pauli and Samec, *Biochem. Z.*, **59,** 470 (1914).
[3] Pauli and Matula, *Kolloid-Z.*, **12,** 222 (1913).
[4] The presence of electrolytes may influence the alcohol flocculation, causing a shift in the pH value of maximum flocculation ; cf. Labes and Zain, *Arch. exptl. Path. Pharmakol.*, **146,** 63 (1929) ; Fenn, *Proc. Nat. Acad. Sci.*, **2,** 534 (1916).
[5] *Trans. Faraday Soc.*, **26,** 723 (1930).
[6] *Rend. accad. sci. Napoli.*, (4), **35,** 171 (1929).
[7] Alexander's " Colloid Chemistry " (New York, 1928), Vol. 2, p. 121 ; *Am. J. Physiol.*, **90,** 291 (1929).
[8] Cf. data by Traube, *Biochem. Z.*, **42,** 470 (1912) ; Windisch and Dietrich, *ibid.*, **97,** 135 (1919) ; **100,** 10 (1919).

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isoelectric point, proteins exhibit minimum surface tension. In other words, " proteins lower the surface tension of solvents to the maximum degree when they are found as undissociated molecules." Naturally, the effect is only observed with those proteins (serum albumin, hæmoglobin and gelatin), which are not precipitated at this point. Isoelectric precipitation, as with globulin and hæmocyanin, removes the protein from solution, with the result that the surface tension would *increase*, tending towards the value for the pure solvent.

ZWITTERIONS

Various workers have suggested that an ampholyte might ionise to form ions of the type $^{+}NH_3 - R - CO^{-}$. Such hermaphroditic ions were named *Zwitter-Ionen*.[1]

These amphoteric ions cannot be demonstrated by cataphoresis. It has been assumed [2] that their concentration is very small and that they play but a slight *rôle* in the equilibrium of the dissociation of amino-acids. Recently, however, considerable interest has been revived in the subject and important theoretical deductions made. Thus, Weber [3] argues that isoelectric proteins are present entirely as practically completely charged zwitterions. Borsook and MacFadyen [4] have closely examined the merits of the classical and zwitterion theories, and presented experimental data confirming the latter, whilst showing no accord with the former.

Comparing the older conception of the isoelectric state of an ampholyte with the zwitterion hypothesis in relation to the action of added acid and alkali, we may represent conditions as follows :—

	Classical.	Zwitterion.
Isoelectric . .	$R\begin{cases}COOH\\NH_2\end{cases}$	$R\begin{cases}COO'\\NH_3^+\end{cases}$
HCl added . .	$R\begin{cases}COOH\\NH_3^+\ Cl'\end{cases}$	$R\begin{cases}COOH\\NH_3^+\ Cl'\end{cases}$
NaOH added. .	$R\begin{cases}COO'\ Na^+\\NH_2\end{cases}$	$R\begin{cases}COO'\ Na^+\\NH_2\end{cases}$

It is to be emphasised that on either theory the addition of acid

[1] Bjerrum, *Z. physik. Chem.*, **104,** 147 (1923). See also Bredig, *Z. Elektrochem.*, **6,** 33 (1899) ; Adams, *J. Am. Chem. Soc.*, **38,** 1503 (1916) ; Michaelis and Mizutani, *Z. physik. Chem.*, **116,** 152 (1925) ; Lewis and Daniel, *Am. Coll. Symp. Monog.*, **4,** 130 (1926).

[2] " Hydrogen Ion Concentration " (London, 1926) .p. 73.

[3] *Biochem. Z.*, **218,** 1 (1930).

[4] *J. Gen. Physiol.*, **13,** 509 (1930).

(or alkali) will give the same end product, but the mechanism involved is the point in dispute. Thus :

	Classical.	*Zwitterion.*
Addition of HCl .	The weakly basic amino group is now able to dissociate.	There is depression of the dissociation of COOH and hence the ionised NH_2 group can combine with the acid to yield a salt.
Addition of NaOH	Permits ionisation of the weakly-acidic COOH.	Ionisation of the NH_2 group depressed, hence COOH combines with the cation of the added base.

As Borsook and MacFadyen emphasise, " the essential difference between the two hypotheses consists in the strengths to be ascribed to the acid and basic groups." Whilst on the classical view these groups are much weaker than acetic acid and ammonia respectively, the zwitterion theory demands that they be somewhat stronger.

At the isoelectric point, the zwitterion theory postulates complete dissociation of both the acid and basic groups. Pauli and his co-workers have devoted much attention to the zwitterion theory, and Pauli [1] has recently given summaries of his views. In the region of the isoelectric point the proteins exist as polyvalent zwitterions and " the polyvalent amphionic structure can condition all the reactions of the proteins with neutral salts, and is also an important factor in the behaviour with other zwitterions." The possibility exists of a mutual competition of the charges of opposite sign on the protein molecule in determining reactions.

The dehydrating influence of alcohol (and, in certain instances, heat) reflects the interdependence of the zwitterionic structure and hydration. Due to the pronounced decrease in the constants of the medium and the dissociation constant, alcohol (or, in some cases, increased hydrolysis under the influence of heat) favours the development of undissociated forms from the zwitterionic form. Increasing the free charge of a protein increases its hydration and alcohol now possesses less effect in precipitation.

Experimental proof of the existence of the zwitterion constitution of the amino-acid molecule has been given, following new technique, by Harris,[2] and the student is referred to his important papers for further details.

Stearn [3] has presented experimental data bearing on the above to the effect that the ampholyte ion of isoelectric gelatin is not chemically inert. His results indicate replacement reactions between dyes and gelatin, acid dyes liberating hydroxyl ion or

[1] *Trans. Faraday Soc.*, **26,** 723 (1930); *Kolloid-Z.*, **53,** 51 (1930). See his previous work, *Biochem. Z.*, **151–152,** 360 (1924); **153–154,** 253 (1924); **155–156,** 482 (1925); **163–164,** 401 (1925); **173–174,** 308 (1926).

[2] *Biochem. J.*, **24,** 1080 (1930); Birch and Harris, *ibid.*, **24,** 1086 (1930).

[3] *J. Phys. Chem.*, **34,** 973, 981 (1930).

using up hydrogen ion, and basic dyes liberating hydrogen ion. The important point observed was that such reactions proceed continuously throughout a pH range, which extended to both sides of the isoelectric point. Although the mechanism resembles a reaction involving displacement of ions, his data indicate that the dyes not only react with free protein ions but with protein in some other form. The latter is considered to be an ampholyte ion, the form in which isoelectric gelatin probably exists.

Before proceeding to the discussion of the important problem of protein stability in solution, it is well to consider briefly the familiar example : egg-albumin.

EGG-ALBUMIN

The colloid chemistry of the albumins is important in relation to food chemistry, particularly so with regard to egg-albumin, used so largely in confectionery, salad dressings, nougat, etc. The interesting problem of the denaturation of proteins is necessarily introduced by any discussion of egg-albumin.

COMPOSITION OF EGGS

Friese [1] gives the following data relating to various eggs :—

Egg.	Average Total Weight.	White.	Yolk.	Shell.
	Grs.	Per cent.	Per cent.	Per cent.
Hen . .	57·12	56·22	31·72	12·06
Goose . .	137·38	53·23	32·72	14·05
Duck . .	78·11	50·03	38·02	11·95
Turkey . .	92·93	56·72	32·51	10·77
Pheasant .	27·03	52·79	37·33	9·88

The analysis of fresh eggs (in per cent.) as determined by Plimmer [2] is :—

	Water.	Ash.	Protein.	Fat.	Undetermined.
White, Hen .	87·2	0·6	10·7	0·10	1·4
,, Duck .	87·5	0·7	10·0	0·03	1·77
Yolk, Hen .	47·1	2·0	15·5	33·30	2·10
,, Duck	45·1	1·5	15·3	36·50	1·60

[1] *Z. Untersuch. Lebensm.*, **46,** 33 (1923). *Vide* also, Plimmer, " Analyses and Energy Values of Foods " (London, 1921), p. 78 ; Hepburn and Katz, *J. Franklin Inst.*, **203,** 835 (1927).

[2] *Loc. cit.*, p. 77.

The dextrose content of the white of hen's egg varies between 0·29 and 0·57 per cent., and of the yolk, between 0·11 and 0·15 per cent.[1] The calcium content of the white is variable, being from 0·59 to 4·25 per cent. of the ash.[2]

EGG-WHITE

At 17° C. water dissolves 15·35 per cent. of egg-albumin, and natural hen's egg-albumin is such a saturated solution with small amounts of fat, salts, etc.[3] Vladimirov [4] found the electrical conductivity to be $7{\cdot}6 \times 10^{-3}$ when fresh, falling to 3×10^{-3} after incubation until the white disappeared. The viscosity of the white decreases with the age of the egg, falling to about 20 per cent. of its original value in a month at ordinary temperature, but requiring about 5 months at 1°—2° C.[5]

Natural egg-white is a mixture of two fluids : (*a*) thin, fairly homogeneous and of syrupy consistency ; (*b*) thick, non-flowing and having a marked tendency to cohere. St. John and Green [6] estimated the thick or gel portion as 54—86 per cent. of the total white, the average of twenty eggs being 68 per cent.

EGG-ALBUMIN

Egg-albumin is a protein with isoelectric point of pH 4·55.[7] Its molecular weight has been determined by many workers and quite different results published. Herzog [8] found values of 17,000 or 73,000 according to the formula used in his diffusion measurements. Sørensen [9] obtained the value 34,000, a result considered by Adair [10] to be about half the " true " value (66,000) By the method of depression of freezing point Takeda [11] obtained the value 1,982, whilst from consideration of the sulphur content he gave 2,133. Marack and Hewitt [12] give 43,000.

The most reliable data is by Svedberg and Nichols,[13] based on a centrifugal sedimentation equilibrium. They found that in moderately pure egg-albumin there was foreign material with a

[1] Hepburn and St. John, *J. Am. Inst. Homeopathy*, **14,** 339 (1921).
[2] Polack and Weber, *Ann.*, (1849), 76 ; Kreis and Studinger, *Schweiz. Apoth. Ztg.*, **59,** 193 (1921).
[3] Rakuzin and Flier, *J. Russ. Phys. Chem. Soc.*, **48,** 458 (1916) ; Straub and Hoogerduyn, *Rec. trav. chim.*, **48,** 49 (1929).
[4] *Biochem. Z.*, **177,** 280 (1926).
[5] Cortese, *Ann. chim. applicata.*, **19,** 260 (1929).
[6] *J. Rheology*, **1,** 484 (1930) ; Washington Agr. Expt. Sta., *Bull.*, **237,** 22 (1930).
[7] *Trans. Faraday Soc.*, **26,** 737 (1930). Other values given are : pH 4·7 [Briggs, *J. Am. Chem. Soc.*, **50,** 2358 (1928)] and pH 4·8 [Sørensen, *Compt. rend. trav. lab. Carlsberg*, **12,** 68 (1917)].
[8] *Kolloid-Z.*, **2,** 2 (1907) ; *Z. Elektrochem.*, **16,** 1003 (1910).
[9] *Medd. Carlsberg Lab.*, **12,** 348 (1917). A later value (35,000) is given in *J. Gen. Physiol.*, **8,** 599 (1927) ; cf. Cohn *et al.*, *J. Biol. Chem.*, **63,** 721 (1925).
[10] *Proc. Camb. Phil. Soc.* (*Biol. Sciences*), **1,** 75 (1924).
[11] *J. Biochem.* (*Japan*), **2,** 103 (1922).
[12] *J. Physiol.*, **66,** v (1928).
[13] *J. Am. Chem. Soc.*, **48,** 3081 (1926).

molecular weight of 170,000, but when this was removed by electrodialysis the true egg-albumin had a molecular weight of 34,500 ± 1,000.

Later work in Svedberg's laboratory [1] has served to clear up the conflicting results just mentioned. By means of his ultracentrifuge technique the pH-stability region of egg-albumin has been explored. In the pH range 4—9, the protein is stable and its molecular weight is constant, the mean value being 34,200. At pH values < 4 and > 9, the protein molecules are split up into a substance which cannot be centrifuged out. When the pH value is < 3 aggregates of denatured protein are formed, whilst at pH > 9 breaking up of the whole material sets in. The molecule of egg-albumin is, according to Nichols and Svedberg, spherical with a radius of $2{\cdot}17\mu\mu$.

Egg-albumin is a typical colloid, having a gold number of 0·08—0·15 (fresh egg-white). In aqueous solution it shows ultramicroscopic particles, the pH at which these are in maximum number being influenced by other ions present.[2] Thus, the maximum is shifted to the acid side by anions : SCN > Cl, Br, I > HPO_4 > SO_4, whilst Ca and Mg shift the maximum to a more alkaline region. The protein lowers the surface tension of water and raises the viscosity. The following data (25° C.) are given by Clark and Mann [3] :—

Per cent. Egg Albumin.	Surface Tension.	Relative Viscosity.
	dynes/cm.	
0·05	70·30	1·014
0·1	68·99	1·024
0·409	66·27	1·054
0·918	61·67	1·057

Over a temperature range of 20°—60° C. the viscosity of a 0·2 per cent. solution remains practically constant,[4] but at higher temperatures coagulation sets in.

CRYSTALLINE EGG-ALBUMIN

Although egg-white can be dried *in vacuo* to a crystalline light-yellow solid and, as such, is a commercial article employed in food preparations, it must not be confused with the crystalline form of the protein described by various research workers.

Sørensen [5] has been a prominent worker in this field. The whites of fresh eggs are treated with an equal volume of saturated

[1] Svedberg, *Trans. Faraday Soc.*, **26,** 737, 740 (1930); Sjögren and Svedberg, *J. Am. Chem. Soc.*, **52,** 5187 (1930); Nichols, *ibid.*, **52,** 5176 (1930).
[2] Schröder, *Biochem. Z.*, **195,** 210 (1928).
[3] *J. Biol. Chem.*, **52,** 179 (1922).
[4] *J. Biochem. (Japan)*, **11,** 423 (1930).
[5] *Medd. Carlsberg Lab.*, **12,** 12 (1917); *J. Am. Chem. Soc.*, **47,** 457 (1925); cf. La Rosa, *Chemist-Analyst*, **16,** (2), 3 (1927).

solution of $(NH_4)_2SO_4$ to precipitate globulin. After filtering, saturated solution of $(NH_4)_2SO_4$ is added until there is an incipient permanent turbidity. This is cleared by addition of $0{\cdot}2N.H_2SO_4$, the solution changing from reddish yellow to yellow. Addition of alkali follows, a voluminous amorphous precipitate being thrown down. The optimum pH value (4·6—4·7) for crystallisation arrives when the precipitate just redissolves on agitation, forming a very opalescent solution which commences to crystallise in one hour.

The system is then filtered and the filter washed with saturated solution of $(NH_4)_2SO_4$, which avoids the formation of a turbid filtrate. The residue is redissolved in water and addition made of saturated solution of $(NH_4)_2SO_4$ until a voluminous amorphous precipitate forms which just redissolves on agitation. Crystallisation again sets in and the cycle of operations is repeated at least three, and preferably six, times. For special work, very pure $(NH_4)_2SO_4$ is recommended, and the egg-albumin is dialysed at 0° C., using well-boiled distilled water and toluene (as preservative). This is continued for one week, when the protein solution is mixed with $N.NH_4.OH$, until the reaction is slightly alkaline to litmus. Another dialysis is made and the process repeated until no further removal of SO_4 ions is possible.

Sørensen believes that " there does not appear to be any reason for considering the crystallisation of egg-albumin to be of a different nature from crystallisation processes in general ; in our opinion, it is simply the crystallisation of a supersaturated solution of a substance crystallising slowly and with difficulty."

The colourless crystals are very refractive rhombic needles and contain 22 per cent. of water.

DENATURATION OF PROTEINS

By a variety of processes, including the action of acids,[1] alkalies, salts,[2] alcohol,[3] heat, radiation,[4] mechanical agitation and other influences, proteins may be converted from soluble into insoluble forms. The general term covering such phenomena is denaturation, although the present tendency is to limit the definition of this. In the present state of knowledge what correspondence there is, if any, between the results obtained by the different processes is not known, but it would seem reasonable to believe that they are fundamentally different. Apparently no change occurs in molecular weight of the protein when denatured.[5] As heat-denaturation has received wide

[1] Cubin, *Biochem. J.*, **23,** 25 (1929) ; Wu and Yen, *J. Biochem.* (*Japan*), **4,** 345 (1924) ; *Chinese J. Physiol.*, **1,** 81 (1927).

[2] Artom, *Arch. sci. biol. Italy*, **14,** 391 (1930).

[3] Booth, *Biochem. J.*, **24,** 1699 (1930).

[4] Spiegel-Adolf, *Strahlentherap.*, **29,** 367 (1928) ; *Biochem Z.*, **213,** 475 (1929) ; Rajewsky, *ibid.*, **227,** 272 (1930) ; Clark, *Am. J. Physiol.*, **73,** 647 (1925).

[5] Huang and Wu, *Chinese J. Physiol.*, **4,** 221 (1930). But see Burt and Greenburg, *J. Biol. Chem.*, **87,** 197 (1930).

attention [1] and has practical significance for the food chemist, it will be treated first.

HEAT-COAGULATION OF EGG-ALBUMIN

When egg-white is heated, as in boiling eggs, it is coagulated. Around this " simple " phenomenon has grown a large literature now developing along distinctly controversial lines. Two major problems present themselves : (1) what is the mechanism involved in the heat-coagulation ? (2) is the process reversible ?

Following Hardy [2] it has been usual to separate the heat coagulation phenomenon into two stages : (1) a preliminary chemical alteration of the protein, (2) a physical change. These stages are respectively denaturation and flocculation.

Considerable speculation has been made concerning the actual chemical change in the denaturation stage. Chemical condensation between free NH_2 and COOH groups of adjacent molecules leading to a re-arrangement of molecular linkages is one theory, simple hydrolysis is another.

The early work of Aronstein [3] and of Heinius [4] showed that electrolyte-free protein did not coagulate on boiling, whereas after cooling the boiled solution and adding NaCl, flocculation occurred. Ostwald [5] observed that the temperature of the coagulation of egg-albumin depended on the concentration of salts present, according to the relation $\frac{1}{t} = Kc^m$, where t = coagulation temperature, c = concentration of electrolyte employed, K and m = constants.

Lepeschkin [6] held that denaturation was a slight hydrolysis of protein not detectable by chemical analysis. There is some difference of opinion on this, Booth [7] as a result of electro-titration experiments finding that the denaturation of egg-albumin by heat did not affect the number of titratable groups, which is contrary to the observations of Wu and Wu,[8] and Wu and Chen.[9]

Regarding the influence of salts, Lepeschkin found that the heat-denaturation of egg-albumin is increased by small amounts of salts, decreased by greater amounts and unaltered with intermediate concentrations of salts. When sufficient salt was present, the

[1] See the pioneer investigation of Chick and Martin, *Kolloidchem. Beihefte*, **5,** 49 (1913–14) and the summaries by Sørensen, *Compt. rend. trav. lab. Carlsberg*, **15** (ix), 1 (1925), and Spiegel-Adolf in Alexander's " Colloid Chemistry," Vol. 2, 303 (1928).

[2] *J. Physiol.*, **24,** 158 (1899).

[3] *Pflüger's Arch.*, **8,** 75 (1874).

[4] *Ibid.*, **9,** 514 (1874).

[5] *Kolloid-Z.*, **2,** 108, 138 (1907). Cf. Pauli and Handowski, *Biochem. Z.*, **24,** 239 (1910).

[6] *Biochem. J.*, **16,** 678 (1922).

[7] *Ibid.*, **24,** 158 (1930).

[8] *J. Biol. Chem.*, **64,** 369 (1925).

[9] *Chinese J. Physiol.*, **3,** 7 (1929).

coagulation of the denatured albumin proceeded at a greater rate than the denaturation process at the same temperature. Acids effected a marked increase and alkalies a marked decrease in the rate of coagulation of denatured albumin.

Prolonged dialysis of egg-albumin produced such change therein that after denaturation the protein is more sensitive to the influence of salt. He concluded that the coagulation of denatured albumin is partly chemical, depending on the chemical nature of the salts present, and partly physical, involving the adsorption of ions.

In a later communication Lepeschkin [1] stated that denaturation is a hydrolysis reaction proportional to the concentration of H and OH ions. Coagulation follows because of union of the albumin with electrolytes, two zones of concentration being effective in this connection : (1) 0·001—0·01 per cent., (2) $>$ 0·1 per cent. Dialysis of egg-albumin for several days resulted in the protein being non-coagulable by heat, whereas three weeks' dialysis gave a heat-coagulable protein, which on dilution with five volumes of water was again not coagulated by heat. Prolonged dialysis rendered the albumin much more sensitive to the influence of electrolytes probably because of the decrease in its dispersity.

Michaelis and Rona [2] have also found that the coagulation of denatured albumin varies with the hydrogen ion concentration, whilst salts exert a 2-fold effect in this connection : (1) anions displace the optimum pH for coagulation to the acid side, and cations to the alkaline side ; (2) there may be weakening or strengthening of the maximum precipitation at the isoelectric point as compared with maximum precipitation in the absence of salts. There is thus competition between other ions and H and OH ions in regard to adsorption by the protein.

Investigations by Lewis [3] led to the conclusion that "the mechanism of the heat denaturation of proteins consisted of a localised hydrolysis of certain links (nature unspecified) situated at various points on a large, heavily hydrated molecular unit." Lewis found that the velocity of the heat-denaturation of egg-albumin in unbuffered solutions at 65° C. and 70·2° C. is not a maximum at the isoelectric point, but that there is a minimum velocity at pH 6·76, where the H and OH ions are sensibly equal in concentration. Since there is a rapid increase in the velocity of denaturation with increasing H or OH ion concentration, on either side of pH 6·76, Lewis assumes that the reaction is catalysed by these ions, *i.e.*, the process is essentially one of hydrolysis.

The recognition of several stages in the general phenomenon of denaturation is now agreed. Cubin,[4] for example, holds that

[1] *Kolloid-Z.*, **31,** 342 (1922).
[2] *Biochem. Z.*, **94,** 225 (1919).
[3] *Biochem. J.*, **20,** 965, 978, 984 (1926).
[4] *Ibid.*, **23,** 25 (1929). Cf. Reiner and Marton, *Kolloid-Z.*, **32,** 273 (1923).

denaturation is the distortion or opening up of the protein unit, whilst flocculation is the process following this and rendered possible by it. Interaction of NH_2 and COOH groups situated on contiguous colloid units leads to aggregation and, hence, coagulation. Logically, any blocking or inactivation of these groups should prohibit coagulation. Accordingly, experiments were made using formaldehyde which, by union with NH_2, yields a methylene-imino linkage. Employing denatured oxyhæmoglobin, Cubin noted that flocculation occurred over a wider pH range in the absence of formaldehyde.

Wu [1] differentiates three stages in the general phenomenon of heat-denaturation : (1) *Denaturation* is the alteration in the normal protein due to the action of acids or alkalies, insolubility being a consequence. The process is slow in the isoelectric region, but is accelerated by heat or alcohol. (2) *Flocculation* is the precipitation of the denatured protein when its solution is brought within the isoelectric zone of the protein in its denatured form. (3) *Coagulation* is the alteration in normal protein as evidenced by decrease in solubility of the normal or the denatured form consequent upon the action of heat, alcohol, or agitation in the isoelectric zone of the protein.

It should be understood that Wu's conception is that coagulation is a distinct process, and not the sum of denaturation and flocculation. It arises possibly from a change in the protein molecule. Any subsequent changes in the rate of combination, adsorption effects and acid- and base-binding capacities are due solely to the fact of the physical change characteristic of coagulation.[2]

Later, Wu and Chen [3] showed that the denaturation of egg-albumin is accompanied by the liberation of non-protein chromogenic substances, whereas coagulation is not.

RE-PEPTISATION OF COAGULATED EGG-ALBUMIN

The possibility of reversing heat-denatured protein is intriguing in view of the general consensus of opinion that the protein has suffered an irreversible change. Modern work points clearly to the possibility of reversing protein coagulation.[4]

Wilheim [5] was able to bring heat-coagulated serum-albumin into solution by the aid of concentrated solutions of certain thiocyanates, salicylates and benzoates. Removal of the salt by dialysis rendered the albumin once more coagulable. He found two fundamental conditions necessary to redissolve the denatured protein : (1) a

[1] *Chinese J. Physiol.*, **3,** 1 (1929).
[2] Wu and Chen, *ibid.*, **3,** 7 (1929).
[3] *Ibid.*, **3,** 75 (1929). Cf. Galinsky, *Biochem. J.*, **24,** 1715 (1930).
[4] See the interesting review by Anson and Mirsky, *J. Phys. Chem.*, **35,** 185 (1931).
[5] *Kolloid-Z.*, **48,** 217 (1929).

minimum salt concentration, (2) a minimum amount of water, depending on the amount of coagulated albumin. This points to the dissolution proceeding in two stages: (1) swelling of the coagulum, the salt concentration controlling same; (2) dispersion of the swollen coagulum, the amount of liquid being an important factor.

Quite recently experiments have been carried out by Bancroft and Rutzler,[1] who found that heat-coagulated egg-white sols are peptised by Kl, KCNS, urea, NH_4CNS and Na_2CO_3, formaldehyde and sucrose. NaOH was also effective, although possibly slight hydrolysis also took place. Some strange results followed the use of ether, which, under ordinary conditions, does not denature egg-albumin. *If the egg-white solution is first extracted by ether, heat-coagulation is nil even at* 100° *C.*

It was shown that ether extracts at least two colloidal materials from egg-white, one positively charged and the other negatively charged, this predominating. The concentrated ether extract coagulates a 10 per cent. egg-albumin sol.

That the coagulated and re-peptised egg-white sols are identical was proved by immuno-biological tests for species specificity, and by the fact that the isoelectric point remains unchanged.

From a practical standpoint, the authors' experiments with sucrose as a means of preventing heat coagulation are decidedly interesting and confirm the observations of Beilinsson.[2]

DENATURATION BY AGITATION

In 1894 Ramsden [3] showed that simple agitation of certain protein solutions brought about a separation of the protein as fibrous or membrano-fibrous solids, "rolled-up and contorted membranes." In this way it was possible to remove all the protein from egg-white solution. The protein is coagulated and is no longer soluble in water. Pre-flocculation of the albumin by electrolytes nullifies the experiment.[4]

The phenomenon is observed with those proteins which are surface-active, *i.e.*, lower the surface tension of water. On thermodynamic grounds it can be shown that the protein will accumulate in the air/liquid interface (see p. 390), and an extension of this interface by shaking to produce a foam brings about an increased adsorption of protein, leading eventually to de-solution.

Wu and Ling [5] have recently studied the problem of surface-

[1] *J. Phys. Chem.*, **35,** 144 (1931).
[2] *Biochem. Z.*, **213,** 399 (1929).
[3] *Du Bois Reymond's Arch. f. Anat. u. Physiol.* (1894), 517; *Proc. Roy. Soc.*, **A72,** 156 (1903); *Z. physik. Chem.*, **47,** 336 (1904); *Trans. Liv. Biol. Soc.*, **33,** 3 (1919).
[4] Jochims, *Biochem. Z.*, **203,** 142 (1928).
[5] *Chinese J. Physiol.*, **1,** 407 (1927).

denaturation in some detail. They state that while heat- or alcohol-denaturation is a sum of processes, mechanical coagulation is a single process. It is decreased by other surface-active materials, which also compete for place in the interface, and is increased by those salts and non-electrolytes, which do not influence the surface tension. Maximum coagulation of egg-albumin was observed at the isoelectric point.

CHAPTER IV

GELATIN

To the colloid chemist gelatin has special interest, it being Graham's original example of a colloid. In technical chemistry it also occupies an important position, and it offers a special field for study by the food chemist. It is used in such diverse preparations as ice-cream, marshmallows, emulsions, jelly tablets and crystals, soups, confectionery, glaze or browning, milk for sick children, etc.

A mass of experimental work has been described in the literature, inasmuch as the chemistry of gelatin reflects immediately on such matters as proteins, jellies, syneresis, etc. The present description is far from exhaustive ; rather does it tend to emphasise the main points in the physico-chemical study of gelatin and to indicate the present trend of ideas. Further, the needs of the technical food chemist are borne in mind.

Gelatin is derived from collagen by hydrolysis,[1] collagen being a scleroprotein contained in the skin, sinews and tendons in animals like the ox, calf and sheep. Collagen is an extra-cellular protein and is very similar to ossein, the protein of bones which can also be hydrolysed to gelatin.

At the onset the student should definitely understand that the term *gelatin* refers not to a real chemical entity, but to a material which differs in behaviour according to its source and manner of production, and which in solution yields different molecular species of varying molecular weights.[2] He should realise, too, the enormous influence of the thermal history of gelatin in relation to its subsequent behaviour.[3]

Gelatin molecules are large [4] and the published molecular weights vary greatly.[5] Recently, Krishnamurti and Svedberg [6] have examined gelatin solutions by the aid of the ultra-centrifuge.

[1] For details of the technical production of gelatin see Alexander, " Glue and Gelatin " (New York, 1923) ; Bogue, " The Chemistry and Technology of Glue and Gelatin " (New York, 1922) ; Smith, " Glue and Gelatin," (London, 1929).

[2] Kraemer and Svedberg, *J. Am. Chem. Soc.*, **52,** 2897 (1930) ; Kraemer, *Am. Coll. Symp. Monog.*, **4,** 107 (1926).

[3] Thimann and Page, *Biochem. J.*, **24,** 1058 (1930).

[4] See Simms, *J. Gen. Physiol.*, **11,** 621 (1928) ; Abitz *et al.*, *Naturwissenschaften*, **18,** 754 (1930).

[5] Paal, *Ber.*, **25,** 1202 (1892) ; Procter, *J. Chem. Soc.*, **105,** 313 (1914) ; Biltz, *Z. physik. Chem.*, **91,** 705 (1916) ; Smith, *J. Am. Chem. Soc.*, **43,** 1350 (1921) ; Wintgen and Vogel, *Kolloid-Z.*, **30,** 45 (1922) ; Schryver, *Biochem. J.*, **17,** 487 (1923) ; Kunitz, *J. Gen. Physiol.*, **10,** 835 (1927) ; Ferguson, *Am. Coll. Symp. Monog.*, **5,** 177 (1928) ; Shito, *Hokkaido J. Med.*, **7,** 187 (1929).

[6] *J. Am. Chem. Soc.*, **52,** 2897 (1930).

Over the range, pH 4·6—6·0, aggregation of the gelatin molecules is pronounced, particularly as the isoelectric point is approached, whilst at pH 4 and pH 7·5 aggregation is practically *nil*, which is in accord with the data from light-scattering measurements. The gelatin sols were revealed as heterogeneous even at pH 4, where there is no aggregation. Various molecular species are present with molecular weights ranging from 70,000 to 11,000. Since there was no appreciable sedimentation in the ultra-centrifuge of gelatin sols at pH 2·5, it is concluded that in such acid solution the gelatin molecules have undergone pronounced decomposition.

The fractionation of gelatin has been reported by several workers.[1] The phenomenon may be distinct from the isolation of protein impurities in gelatin, such as the heat coagulable protein [2] in gelatin prepared from calf-skins. This occurs to the extent of less than 1 per cent., has an isoelectric point of pH 4·0, and is suggested as being identical with the so-called " insoluble " gelatin mentioned by various investigators (see p. 79). The true explanation of " fractionation " still remains to be given.

ASH-FREE GELATIN

For the purposes of investigation, purified gelatin is frequently desirable and methods of de-ashing gelatin have been developed. Loeb [3] pointed out that to remove ionogenic impurities gelatin should be brought to the isoelectric point, where it " must give off any ion with which it was combined." To prepare such isoelectric gelatin he gives the following directions [4]: 50 gr. commercial powdered gelatin are stirred into 3,000 c.c. M/128 acetic acid at 10° C., and after 30 minutes the supernatant liquor is decanted and replaced by fresh M/128 acetic acid at 10° C., until the original volume is restored. Stir frequently, and after 30 minutes again decant and replace by an equal volume of cold water (5° C.). After thorough stirring, filter by suction in a Buchner funnel through towel cloth. Five successive washings are made on the funnel with 1,000 c.c. lots of water at 5° C. Draining being complete, the gelatin is transferred to a beaker and melted at 50° C. Loeb thus reduced the ash content of his gelatin, so that 100 c.c. of a 1 per cent. gelatin solution gave no more than 1 mgm. of ash. Further washing of the gelatin could remove even this slight ash.[5] Smith [6] also described a method of de-ashing, employing solutions of NaCl and HCl, finally washing with water and then 90 per cent. alcohol.

[1] Rakuzin, *Chem.-Ztg.*, **48,** 249 (1924); Chevallier, *Compt. rend. soc. biol.*, **97,** 484 (1927); Kunitz and Northrop, *J. Gen. Physiol.*, **10,** 167 (1926—7); **12,** 379 (1928).
[2] Sheppard, Hudson and Houck, *J. Am. Chem. Soc.*, **53,** 760 (1931).
[3] *J. Gen. Physiol.*, **1,** 238, 253 (1918).
[4] " Proteins and the Theory of Colloidal Behaviour " (New York, 1924), 41.
[5] Miss Field, *J. Am. Chem. Soc.*, **43,** 667 (1921).
[6] *Ibid.*, **43,** 1350 (1921).

Northrop and Kunitz [1] improved on Loeb's technique : 1,000 gr. powdered gelatin (80 mesh) is soaked for 1 hour in 20,000 c.c. M/128 acetic acid at 5°—10° C., stirring continually. After three washings with cold distilled water, the gelatin is suspended for 1 hour in M/50 NaOH. Again follow three washings in cold distilled water. Acetic acid is now added to bring the system to pH 4·7. The gelatin, swollen by alkali, now shrinks, and further four or five washings with cold water remove electrolytes. The gelatin is then stirred with 5,000 c.c. of 95 per cent. alcohol for 15 minutes, filtered on a Buchner funnel, washed twice with fresh alcohol and finally with ether.

Miss Lloyd [2] purifies gelatin by dialysis at pH 4·6, followed by precipitation with alcohol. The dry gelatin so obtained had an ash of 0·06—0·0 per cent. The gelatin was a snow-white, fibrous, brittle solid, and its maximum solubility in boiling water was only 2·5 per cent.

Sheppard, Sweet and Benedict [3] electrolysed a 5 per cent. gel, using a gold anode and mercury cathode. Electrolysis was conducted below 40° C. for 2—3 weeks. The gelatin was then precipitated by acetone and the excess acetone removed at 70° C. The gelatin, air-dried on netting, gave a yield of 75—80 per cent., and possessed 0·02—0·03 per cent. ash. Johlin [4] electrolyses a 10 per cent. gel in an apparatus made solely of quartz and platinum. Acid concentrates in the water near the anode, the bases remaining chiefly in the gel adjacent the cathode. The ash-free gelatin is precipitated by acetone.

Recently, electrodialysis of gelatin has been employed with successful results in the de-ashing of gelatin.[5]

THE ISOELECTRIC POINT OF GELATIN

Owing to the importance of gelatin in the work of the food chemist, it seems of interest to examine the diversities of opinion regarding its isoelectric point. Different investigators have published widely differing pH values for the isoelectric point. Thus, 4·0,[6] 4·7,[7] 4·75,[8] 4·8,[9] 4·90,[10] 4·95,[11] 5·05,[12] 5·25,[13] 5·27,[14] 5·5,[15] 5·6,[16] 7·3,[17] are all recently determined values.

[1] *J. Gen. Physiol.*, **11,** 477 (1928) ; **9,** 354 (1925—6).
[2] *Biochem. J.*, **14,** 147, 584 (1920) ; **16,** 530 (1922).
[3] *J. Am. Chem. Soc.*, **44,** 1857 (1922).
[4] *J. Phys. Chem.*, **29,** 274 (1925). This is an improvement on the method of Knaggs *et al.*, *Biochem. J.*, **17,** 473 (1923) ; **18,** 1079 (1924), and Manning, *ibid.*, **18,** 1085 (1924).
[5] Dhéré, *Kolloid-Z.*, **41,** 315 (1927) ; *Bull. soc. chim. biol.*, **8,** 604 (1926) ; Shumpeioka, *J. Chem. Soc. Japan*, **30,** 396 (1927).
[6] Vanlehrberghe, *Bull. assocn. élèves inst. sup. fermentations Gand.*, **28,** 332 (1927).
[7] Rawling and Clark, *J. Chem. Soc.*, **121,** 2830 (1922) ; Jermolenko, *Kolloid-Z.*, **48,** 143 (1929).
[8] Gerngross and Bach, *Collegium* (**1922**), 350.

[9]—[17] For notes, see page 66.

The isoelectric point of gelatin varies with its origin and its purity. Thus, Patten and Kellens [1] reported the isoelectric point of a commercial gelatin as pH 5·64, but the ash-free gelatin had an isoelectric point at pH 4·8, confirmed by Rawling and Clark,[2] who found the minimum viscosity of electrophoretically-prepared gelatin, in the presence of sulphuric or acetic acid, was at pH 4·7 at 35·4° C. Again, Gerngross and Bach [3] found values from pH 4·45 to pH 5·0, and gelatins prepared from three samples of hide powder had isoelectric points at pH 5·55, pH 5·4, and pH 5·51 respectively. They [4] also observed values of pH 5·0 and pH 5·5 for bone gelatin and pure hide gelatin respectively.

The absorption spectrum of gelatin has been studied by Higley and Matthews,[5] investigating the relation between the wave-length of maximum absorption in the ultraviolet and the pH values of 1 per cent. gelatin sols. On plotting the wave-length as ordinates against pH value as abscissæ, the authors found two points of maximum absorption, at pH 3·5 and pH 6·6, and two minima, at pH 4·69 and 7·65. Whether the higher pH values refer to a second isoelectric point is left an open question. A curious feature is the sharp shift of the absorption band towards the ultraviolet as the pH values 4·69 and 7·65 are approached.

Kraemer and Dexter,[6] assuming that the Tyndall effect has a maximum at the isoelectric point, found that gelatins of various origins possess widely differing pH values for the isoelectric point, *e.g.*, pH 8 for a pigskin gelatin. Mixtures of different gelatins did not reveal the maxima of the individuals, *i.e.*, double maxima are not observed. The important work of Svedberg [7] on the ultra-centrifugal study of gelatin solutions proves that gelatin is very complex and is not a single chemical species. The statement by Kraemer and Dexter that the " fluctuation in isoelectric point suggests that ' gelatin ' consists of an unknown number of unknown chemical entities " has proved true.

A recent theory due to Johlin [8] is that the minimum physical

[9] Johlin, *J. Phys. Chem.*, **29,** 275 (1925).
[10] Sheppard and Houck, *ibid.*, **34,** 2187 (1930).
[11] Kraemer, *Am. Coll. Symp. Monog.*, **4,** 103 (1926).
[12] Hitchcock, *J. Gen. Physiol.*, **12,** 495 (1929).
[13] Shukoff *et al.*, *J. Russ. Phys.-Chem. Soc.*, **58,** 639 (1926).
[14] Shukoff *et al.*, *ibid.*, **59,** 1061 (1927).
[15] Kraemer, *loc. cit.*, p. 105.
[16] Shukoff *et al.*, *J. Phys. Chem.*, **29,** 285 (1925).
[17] Dahlberg *et al.*, *Ind. Eng. Chem.*, **20,** 397 (1928).

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[1] *J. Biol. Chem.*, **42,** 363 (1920).
[2] *J. Chem. Soc.*, **121,** 2830 (1922).
[3] *Biochem. Z.*, **143,** 542 (1923).
[4] *J. Am. Leather Chem. Assocn.*, **19,** 262 (1924); *Collegium* (**1924**), 1—10. Cf. Carpenter *et al.*, *Ind. Eng. Chem.*, **20,** 397 (1928).
[5] *J. Am. Chem. Soc.*, **46,** 852 (1924).
[6] *J. Phys. Chem.*, **31,** 764 (1927); *Am. Coll. Symp. Monog.*, **4,** 107 (1926).
[7] *J. Am. Chem. Soc.*, **52,** 2897 (1930).
[8] *Proc. Soc. Exptl. Biol. Med.*, **26,** 702 (1929); *J. Biol. Chem.*, **86,** 231 (1930).

properties commonly defining the isoelectric point of gelatin are the properties of pure (de-ashed) gelatin, and pure gelatin is not isoelectric. He argues that pure gelatin has a pH value approx. 4·95, the gelatin possessing at least two isoelectric points, viz., at pH 4·68 and pH 5·26. He believes that isoelectric gelatin is an ionised salt of gelatin with an exact balance of the positive and negative charges on the gelatin radicle, the compensation of the charges being external and due to equal numbers of positive and negative ions of foreign electrolytes.

In discussing the two isoelectric points, Johlin suggests that "the isoelectric point is of an indeterminate nature," the experimental methods employed yielding results relative to the conditions applying in the determination.

The earlier work of Wilson and Kern [1] on the swelling of gelatin led them to the conclusion that gelatin had two isoelectric points, at pH 4·7 and pH 7·7, corresponding to Smith's gel form B and sol form A respectively. Other investigators [2] have also reported dual isoelectric values, and the suggestion has been made that gelatin is a mixture of two substances with isoelectric values at pH 4·7 and pH 8 respectively.[3] Whilst Wilson and Kern's *theory* has been adversely criticised,[4] no satisfactory explanation is yet forthcoming concerning the inflexion point at high pH value in curves connecting certain physical properties with the pH value of gelatin solutions.

Recently, Briefer [5] has published data on the photometric and electrometric measurements of gelatin behaviour which, if confirmed, will throw considerable light on both the varying isoelectric points of different gelatins and the finding under certain conditions of dual isoelectric points. It is shown that no matter what the origin, all gelatins possess similar physical properties and behaviour towards electrolytes "when the initial chemical treatment is of the same order." The fundamentally important statement is made that conditioning the raw material with lime yields a gelatin with isoelectric point near pH 5, independent of the washing out or not of the lime or its removal with acid at any pH value. Should the same raw materials, however, be originally conditioned with acid, the resulting gelatins will have isoelectric points in the zone, pH 7—8. The main characteristics of either type of gelatin will

[1] *J. Am. Chem. Soc.*, **44,** 2633 (1922); **45,** 3139 (1923). See the criticism by Hitchcock, *J. Gen. Physiol.*, **6,** 457 (1924).

[2] Higley and Matthews, *J. Am. Chem. Soc.*, **46,** 852 (1924). See also Sheppard and Sweet, *Ind. Eng. Chem.*, **16,** 593 (1924); Atkin and Douglas, *J. Am. Leather Chem. Assocn.*, **19,** 528 (1924).

[3] Rawling and Clark *J. Chem. Soc.*, **121,** 2830 (1922); Shukoff and Stschoukareff, *J. Phys. Chem.*, **29,** 294 (1925).

[4] Kraemer, *J. Phys. Chem.*, **29,** 410 (1925); Bogue and O'Connell, *J. Am. Chem. Soc.*, **47,** 1694 (1925). Sheppard and Houck, *J. Phys. Chem.*, **34,** 2190 (1930), were unable to confirm Wilson and Kern's second isoelectric point at pH 7·7.

[5] Briefer, *Ind. Eng. Chem.*, **21,** 270 (1929), gives experimental evidence to show that "gelatin may be de-ashed without reference to its isoelectric point."

survive de-ashing of the gelatin. Thus, the chemical treatment of the raw material and not its biological origin determines the physical characteristics.

Accepting maximum turbidity as coincident with the isoelectric point, Briefer found gelatins extracted from limed stock to have isoelectric points, singly or in mixtures, at pH 5. Similarly, gelatins from originally acid-treated stock showed maximum turbidity at about pH 8. On mixing these two types the maximum turbidity occurred between pH 4·7 and pH 8, " the precise location bearing some simple ratio to the proportions of the mixture, but the minimum physical properties will not always be found at the isoelectric point as in the case of straight-run gelatins."

Incomplete liming or acid-plumping of the animal skin results in a gelatin which will have its isoelectric point shifted to an extent depending on the degree of conversion. Again, skins only partly limed and subsequently treated with acid yield gelatins with the isoelectric point corresponding to a mixture of the two types of gelatin.

Sheppard and Houck [1] repeated some of Briefer's tests with lime-treated and acid-treated pigskin gelations. Maximum turbidity gave the isoelectric point between pH 4·9 and 5·0 for the lime-treated pigskin gelatin, but in the case of the acid-treated sample there was no sharp maximum turbidity, but a broad maximum zone from pH 6·8 to 8·3 (another sample, pH 6·0 to 7·3). However, the determination of the isoelectric point by alcohol precipitation gave definite values of pH 4·9 for the lime-treated pigskin gelatin, and pH 4·85 and pH 4·72 for the two samples of acid-treated pigskin gelatin.

THE GOLD NUMBER OF GELATIN

As a measure of the protective action of colloids, Zsigmondy [2] proposed the determination of the *gold number*, *i.e.*, that number of milligrams of the colloid in question which just fails to prevent the colour change to violet of 10 c.c. standard red gold hydrosol on the addition of 1 c.c. of a 10 per cent. solution of NaCl.

It seemed legitimate to ascertain whether the gold number would be characteristic of gelatins of various grades and whether there would be a connection between protective action and other properties particularly the important one of jelly strength. However, experiment shows that the gold number is useless for grading gelatins.

Elliott and Sheppard [3] examined seventeen samples of widely different grades of gelatin, exhibiting gold numbers from 0·005 to 0·015. In agreement with Menz it was found that protective action

[1] *J. Phys. Chem.*, **34,** 2187 (1930).
[2] *Z. anal. Chem.*, **40,** 687 (1901).
[3] *Ind. Eng. Chem.*, **13,** 699 (1921).

increases with concentration of gelatin (gold number decreases). Menz [1] believed this arose from an alteration in the degree of dispersion on dilution larger particles possessing a much weaker protective action. Elliott and Sheppard verified this, observing that the amicrons increase in proportion as the concentration decreases. Ageing the gelatin solution increases the gold number, probably because the amicrons form larger aggregates of less protective power.

Employing six gelatins including high and poor grades, Moore, Combs and Dahle [2] were unable to find any relation between their gold numbers, pH of 0·78 per cent. solutions, and the gel strength. Their results are shown in Table 11.

TABLE 11.

Gelatin.	Gold No.	pH	Gel. Strength.	Ash.
A	0·0040	6·00	82·7	0·793
B	0·0048	5·65	82·0	1·829
C	0·0200	4·81	74·0	1·278
D	0·0130	5·62	74·3	2·819
E	0·0170	4·33	81·0	0·790
F	0·0230	3·86	84·3	0·964

Similar conclusions were reached by Serex and Goodwin.[3] An interesting study has been made by Tartar and Lorah [4] of the influence of pH on the gold number of a gelatin. They find that in the pH zone 5 to 8, the protective action is unchanged, whereas at pH $<$ 4·7 (isoelectric point) there is a rapid decrease of protective action as the hydrogen ion concentration increases. Thus, gelatin only exerts a pronounced protective action for the gold sol when it is isoelectric or possesses the same sign as the gold, *i.e.*, negative.

GELATIN AS AN EMULSIFYING AGENT

It is natural to pass from a consideration of the protective action of gelatin to the question of its efficiency as a stabiliser for emulsions. That gelatin is a useful emulsifying agent has been observed by Briggs and Schmidt,[5] Holmes and Child,[6] Nugent,[7] Clayton,[8] Kernot and Braggs [9] and others.[10]

[1] *Z. physik. Chem.*, **68,** 129 (1909).
[2] *J. Dairy Sci.*, **8,** 500 (1925).
[3] *Ind. Eng. Chem.*, **19,** 473 (1927).
[4] *J. Phys. Chem.*, **29,** 792 (1925).
[5] *Ibid.*, **19,** 484 (1915).
[6] *J. Am. Chem. Soc.*, **42,** 2049 (1920).
[7] *Trans. Faraday Soc.*, **17,** 703 (1922).
[8] *Kolloid-Z.*, **28,** 202 (1921).
[9] *J. Soc. Chem. Ind.*, **47,** 96 T (1928).
[10] Cf. Woodman, *J. Phys. Chem.*, **30,** 658 (1926) ; *J. Pomology Hort. Sci.*, **4,** 184 (1925).

Limburg [1] investigated the relation between the pH value of gelatin solution and its efficiency as an emulsifier for pure paraffin oil. Measurements were made of the cataphoretic velocity of the oil globules at pH values of 2·8, 4·7, 5·5 and 9·6, as a function of the concentration of the gelatin. When sufficient gelatin is present, a steady minimum value is reached for the cataphoretic velocities. At pH = 4·7, the isoelectric point, a zero value obtains. One can assume, therefore, that the oil globules are enveloped with a gelatin layer. At low concentrations of gelatin this layer is incomplete. For complete envelopment of the oil globules, the gelatin concentration required is a function of the pH value, increasing with increasing pH values. Approximately the relations are :—

pH 2·8	requires	0·00064	gr./litre.
pH 4·7	,,	0·00125	,,
pH 5·5	,,	0·01	,,
pH 9·6	,,	0·1	,,

The stability of the emulsions with varying gelatin content is a function of pH. Plotting the oil content of the emulsions as ordinates against gelatin concentration as abscissæ, the curves for systems of different pH values, all show a minimum, the sharpness being a function of pH. The more acid the system, the steeper is the minimum. The electric charge on the globules is the real factor determining the stability. For solutions with pH $< 4{\cdot}7$ the charge is fixed. The gelatin concentration at which the charge is zero, coincides with that at minimum stability, so that instability of the emulsion must be ascribed to the discharge. In solutions with pH $> 4{\cdot}7$, although the first additions of gelatin cause a decrease in the charge, the sign always remains negative. Hence, the minimum stability of the emulsion represents the balance between the diminution of the charge and the protective enveloping of the oil globules, the latter soon conferring marked stability.

Different workers have reported varying results as to the efficiency of gelatin in stabilising emulsions, and this must be attributed in part to a non-recognition of the influence of the pH value of the system, and in part to the diversity of grades of gelatin employed. More and more it is being realised that the biological origin and the chemical preparation of gelatins profoundly affect the physical properties.[2]

Kernot and Knaggs [3] have employed gelatins of various grades to emulsify different oils, and although their use of the Donnan pipette to determine emulsifying efficiency can only lead to fairly

[1] *Rec. trav. chim.*, **45,** 885 (1926). *Vide* Clayton, "The Theory of Emulsions and their Technical Treatment" (London, 1928), p. 88 *seq.*

[2] *Vide* Briefer, *Ind. Eng. Chem.*, **21,** 266 (1929).

[3] *Loc. cit.*

approximate results, they showed that the emulsifying power of the gelatin depends considerably on the nature of the precursor and its chemical treatment. Highly purified gelatins from fish or mammal skins are better emulsifiers than the impure samples. Again, those gelatins are the more efficient which have been derived from alkali-treated precursors.

THE SWELLING OF GELATIN

When gelatin is placed in cold water, imbibition takes place. The " solubility " of gelatin is very slight, data due to Fairbrother and Swan [1] indicating 0·02 gr. per 100 gr. water at 0° C., and 0·10 gr. at 22° C. These authors observed that in HCl, H_2SO_4, HNO_3, acetic acid, KOH and NaOH the " solubility " varied from 0·2 to 5,000 millimols per litre, passing through a minimum in the case of acids. In sufficiently concentrated solutions the gelatin completely dissolved. Neutral salts depressed the " solubility " in the approximate order of the Hofmeister series. Chiari [2] pointed out the influence of impurities on swelling, and modern work is always carried out under carefully noted conditions.

According to Miss Shreve,[3] during the absorption of water by gelatin, the ratio : $\frac{\text{increase in weight of jelly}}{\text{increase in volume}}$, is a constant. The swelling varies with the relation of the quantity of swelling substance to the volume of the liquid, the effect being more pronounced the higher the temperature. Ostwald and Kestenbaum [4] have made a detailed investigation of this solid phase influence in swelling and find that one can express the dependence of swelling on the quantity of solid present by the Kroeker [5] adsorption curve, or by the usual adsorption isotherm, assuming the imbibition of water to be adsorption. The influence of the solid phase is dependent upon the $CaSO_4$ content of the gelatin or the decomposition products present. Miss Lloyd,[6] in her study of the swelling of purified (isoelectric) gelatin in water, acids and salt solutions, found that swelling was independent of the volume of the liquid so long as there is no hydrolysis. In alkalies, slight hydrolysis takes place and swelling increases with increase in the volume of the liquid. The fact that commercial gelatins swell in relation to the volume of the liquid is connected with their content of electrolytes and other impurities.

Northrop and Kunitz [7] investigated the swelling of gelatin added to different volumes of acid at different concentrations at 5° C. In

[1] *J. Chem. Soc.*, **121,** 1237 (1922).
[2] *Biochem. Z.*, **33,** 167 (1911).
[3] *J. Franklin Inst.*, **187,** 319 (1919).
[4] *Kolloidchem. Beihefte*, **29,** 1 (1929) ; cf. Kuhn, *ibid.*, **14,** 147 (1921).
[5] *Kolloid-Z.*, **43,** 252 (1927).
[6] *Kolloid-Z.*, **48,** 342 (1929) ; **54,** 46 (1931).
[7] *J. Gen. Physiol.*, **12,** 537 (1929) ; cf. Kunitz, *ibid.*, **13,** 565 (1930).

the case of isoelectric gelatin the swelling was independent of the volume of acid, varying only with the concentration of the supernatant solution at equilibrium, whereas with unpurified gelatin, *i.e.*, containing neutral salts, the swelling depends also on the volume of the acid. The authors predict these results from the Procter-Wilson-Loeb theory of swelling and show that the conclusions of Küntzel [1] must be reconsidered, since his studies dealt with unpurified gelatin.

According to Buchner,[2] when gelatin swells to its maximum extent in 0·01 N.HCl, or NaOH from 30 to 50 times its weight of liquid is imbibed. Such swollen gelatin is stated to lose water when it is exposed to saturated water vapour at the same temperature, in proportion to its surface, not its mass. The significance of this observation is not clear.

Classical work on the swelling of gelatin was carried out by Arisz,[3] who immersed sheets of gelatin in water at various temperatures. Above 30° C. the swelling is more rapid, and at higher temperatures solution is rapidly effected. At 2° C. and at 12° C. the graphs show that swelling is intense during the first hour [4]; thereafter, imbibition is slow and has not reached equilibrium even after three weeks. The rate of swelling varied with the thickness of the gelatin and with its water content. Using gels, it was found that imbibition varied greatly with the age of the gel, and one could have two gels of the same concentration with greatly different swelling rates. Arisz believed that during swelling, the size of the gelatin particles changes, and the attraction of the particles for each other diminishes. He concluded that swelling involved two stages: in the first, swelling is very rapid and attains a maximum, whilst the second is concerned with certain equilibria changes depending on the internal structure of the gel.

Maximum swelling remains unproved and, indeed, the imbibition of water increases with the degree of dispersion of the gelatin.[5]

The experimental data of Sheppard and McNally [6] show that at and below 15° C., ash-free gelatin exhibits strictly limited swelling in distilled water, equilibrium being rapidly attained. At higher temperatures, swelling progresses more and more, the jelly breaking into ever smaller pieces in excess of water. Such "unlimited swelling" even occurs below the usually accepted transition temperature for sol $\rightleftharpoons$ gel. Apparently, swelling of the jelly is tending to a limiting point (a specific dispersion limit), which is most

[1] *Kolloid-Z.*, **40,** 264 (1926).
[2] *Rec. trav. chim.*, **48,** 1047 (1929).
[3] *Kolloidchem. Beihefte*, **7**, 42 (1915); see abstract of this paper and comments by Procter, *J. Am. Leather Chem. Assocn.*, **12,** 536; **13,** 117 (1918).
[4] See equation by Pauli, *Pflüger's Arch.*, **67,** 219 (1897), which shows that swelling velocity is a maximum at the beginning of imbibition.
[5] Brotman, *J. Soc. Leather Trades' Chem.*, **5,** 226 (1921).
[6] *Am. Coll. Symp. Monog.*, **7,** 17 (1930).

probably a function of the concentration. Under certain conditions of temperature and thickness of the gelatin sheet, an initial contraction is observed. Such contraction was the greater the thicker the sheet and the lower the temperature. The phenomenon is connected with residual strains in the dried sheet of gelatin, swelling being most rapid in directions at right angles to those strains left on drying. Such strains have their origin in the orientation (during drying) of the molecular aggregates in the gelatin.

Shreve [1] emphasises the important part played by the thermal history of gelatin gels in the imbibition or the loss of water. Thus, two gels of similar concentration prepared by (*a*) evaporation of a dilute gelatin solution ; (*b*) evaporation of a more concentrated solution, will show different imbibition capacities, the second being inferior to the first. Newly-cut cubes of gel when placed in water, show equal rates of increase along all three axes, but if evaporation has previously been permitted, the increase is much more pronounced on the dimensions perpendicular to the surfaces where evaporation occurred. These findings are confirmed by Sheppard and Elliott,[2] who postulated a " case-hardening " during drying, a conclusion contradicted by Gortner and Hoffman.[3]

Previous work by the last-named authors [4] showed that gelatin gels of varying concentration, after drying take up water according to their *original water content* and not in relation to surface area. Thus, a 10 per cent. and a 40 per cent. gel were dried to less than 3 per cent. water content, and after remaining 72 hours in water they had imbibed 6·45 gr. and 4·3 gr. water per gr. dry gelatin respectively. They concluded that the gels had a structure which persisted during the drying out and influenced the later re-imbibition. Their next investigation employed uniform size particles of gelatin, passing a 2 mm. sieve but remaining on a 1 mm. sieve, from which solutions were prepared and dried as a gel or as a sol, the former in an air-current below the melting-point and the latter in an oven at 45° C. Whilst particles prepared from a 2·5 per cent. gelatin sol commenced to solate after 6 hours when placed in water, similar particles from a 5 per cent. gelatin gel did not solate even at 24 hours. In general, the gelatin dried as gel takes up the more water. Apparently dried gelatin preparations reflect their thermal history to a considerable degree and the contrast between the behaviour of the sol and gel preparations is to some extent due to the presence of a persistent gel structure. In this connection we recall Smith's [5] belief that gelatin exists in two forms, the sol form A, stable above 33°—35° C., and the gel form B, stable below 15° C. (see p. 96).

[1] *Science*, **48,** 324 (1918).
[2] *J. Am. Chem. Soc.*, **44,** 373 (1922).
[3] *J. Phys. Chem.*, **31,** 464 (1927).
[4] *Proc. Soc. Exptl. Biol. Med.*, **19,** 252, 257 (1922).
[5] *J. Am. Chem. Soc.*, **41,** 135 (1919).

Sols dried above 35° C. and gels dried below 15° C. will yield different solid forms.

More attention has been given to the swelling of gelatin in water under the influence of acids and alkalies.[1] Ostwald [2] observed that imbibition varied with the concentration of acid or alkali, being a minimum in N/210 HCl and N/100 KOH, and a maximum in N/40 HCl and N/36 KOH. Chiari's [3] work showed the importance of the hydrogen ion concentration prevailing, and he suggested that minimum swelling of gelatin occurs at the isoelectric point, an observation confirmed by Wilson and Kern,[4] who, however, noted two points of minimum swelling, viz., pH 4·7 and pH 7·7, irrespective of whether the gelatin had been rendered ash-free or not [5] (see p. 67).

Fischer [6] found that gelatin dissolves progressively in mixtures of salts of polybasic acids when the concentration of acid or alkali in the mixtures increases. Highest swelling occurs in pure acid, decreases to a minimum (*e.g.*, in monosodium phosphate) and rises again to a maximum in alkali. Similarly, Patten and Johnson [7] found that a 3 per cent. gelatin gel liquefies at pH 8·4—10·1 ; least swelling was observed at pH 4·7—5·7. Bogue [8] investigated the swelling and viscosity of gelatin under the influence of sodium silicate, wherein the ratio $Na_2O : SiO_2$ varied from 1·4—1·1. The gelatin showed maximum swelling and viscosity at pH 8·5. The consistency of the jelly, although a solid between pH 4·7 and pH 8·0, is semi-solid at pH 8·5 and liquid at pH 9, refusing to set at 10° C. The effects were more pronounced with isoelectric gelatin, since the retarding action of calcium ions has been removed.

Salts influence the swelling of gelatin, and herein lies the starting point of a modern controversy. Hofmeister placed the effect of the sodium salts on swelling in the order of decreasing activity as : bromides > nitrate > chlorate > chloride > acetate > tartrate > citrate > sulphate. Measuring the swelling of isoelectric gelatin in solutions of 1 N. to 0·1 N. sodium salts, Stiasny and Gupta [9] find the order of decreased swelling effect to be sulphocyanate > nitrate > chlorate > chloride > sulphate, a confirmation of Hofmeister's series. The same authors further showed [10] by ultra-filtration, mutarotation, viscosity, dialysis and other tests that the sodium salts increased the dispersion of isoelectric gelatin. The

[1] Hofmeister, *Arch. exptl. Path. Pharmakol.*, **27,** 395 (1890) ; **28,** 210 (1891) ; Spiro, *Hofmeister's Beiträge*, **5,** 276 (1904).
[2] *Pflüger's Arch.*, **108,** 563 (1905).
[3] *Biochem. Z.*, **33,** 167 (1911).
[4] *J. Am. Chem. Soc.*, **44,** 2633 (1922).
[5] *Ibid.*, **44,** 3139 (1922).
[6] *Science*, **46,** 189 (1917) ; *J. Am. Chem. Soc.*, **40,** 272, 292, 303 (1918).
[7] *J. Biol. Chem.*, **38,** 179 (1918).
[8] *Ind. Eng. Chem.*, **14,** 32 (1922).
[9] *Collegium*, (1925), 13—33.
[10] *Ibid.* (**1925**), 23—36.

work of Lenk [1] suggests that the Hofmeister series is true for molecular concentrations of the sodium salts, but is reversed in dilute solutions. Further confirmation of the Hofmeister series in relation to the swelling of gelatin has recently been provided by von Moraczewski and Grzycki.[2]

Loeb [3] devoted much attention to the question of the reality of the Hofmeister series of ion effects and believed they were not valid because " the older authors made the mistake of not measuring the pH of the gel and hence mistook the effect of differences in the pH of the gel for differences in the chemical nature of the anion of the acid." He argued that the degree of swelling of gelatin was influenced by the valency and not by the chemical nature of the ion in combination of gelatin. However, several authorities have criticised Loeb's views, a discussion of which is given elsewhere (p. 44).

The system : gelatin — acid or alkali $\pm$ NaCl, has been investigated in respect to swelling phenomena by Lloyd and Pleass,[4] over a temperature range 0°—24° C., a pH range 0·5—12, and a concentration of NaCl of 0—2·0 mols. per litre, always having 100 c.c. fluid per 0·1 gr. gelatin. The gelatin was de-ashed to 0·05—0·02 per cent. ash, calculated on the dry weight at 100° C. In the absence of NaCl, the swelling curve shows a minimum at the iso-electric point (pH 5·0), maxima at pH 2·6 and pH 9·8, and a broad maximum between pH 7·5—7·9. Solution of the gelatin is effected at pH $< 1{\cdot}1$ or $> 11{\cdot}6$. Sodium chloride suppresses the swelling on the acid side and at a concentration of this salt in excess of 0·5 M. coagulation of the gelatin results.

A comprehensive study of the swelling of gelatin in fifty organic acids has been made by Kuhn.[5] Except with the slightly soluble acids, maximum swelling occurred in moderate concentration of acid, and Kuhn suggested that such maximum swelling is an equilibrium phenomenon of four simultaneous reactions : (1) true swelling or hydration, (2) peptisation to hydrosol, (3) hydrolysis (chemical decomposition), (4) dehydration (flocculation). Maximum swelling takes place when (1) is overcome by (2) and (3) as the acid concentration increases. There was no relation between maximum swelling and the dissociation constant of the acid employed, although the concentration of the acid at such swelling point is a function of the dissociation constant. The author failed to establish whether there was chemical combination or adsorption involved in the effect of acid and maximum swelling.

[1] *Biochem. Z.*, **73,** 15 (1916).
[2] *Ibid.*, **221,** 331 (1930).
[3] " Proteins and the Theory of Colloidal Behaviour " (New York, 1924), p. 119 *seq.*, p. 370.
[4] *Biochem. J.*, **21,** 1352 (1927). See also the comprehensive paper based on 16,000 measurements by Rudsit, *Kolloid-Z.*, **53,** 205 (1930).
[5] *Kolloidchem. Beihefte*, **14,** 147 (1921).

Confirmation of Kuhn's results has been given by Sauer and Kleverkaus,[1] who investigated the swelling of powdered gelatin in solutions of inorganic and organic acids by the volume method of Fischer,[2] using continuously renewed liquid for the swelling process. The maximum swelling for strong acids lies in a low concentration range, whereas for feebler acids the concentration range is higher. Owing to the use of fresh liquid during swelling, the swelling maxima are at lower acid concentrations than previously indicated in the literature. The unit of weight of swollen gelatin containing acid, rises approximately uniformly with the acid concentration of the external liquid, and is completely independent of the corresponding swelling curve.

Whilst the total concentration of acid in the swollen gelatin is consistently greater than that in the external solution, the reverse is true in respect to pH. The attainment of swelling maxima for a series of acids shows a striking connection between swelling maximum and pH, independent of the degree of dissociation of the acids, results quite contrary to the earlier findings of McLaughlin.[3] The authors summarise their data in Table 12.

TABLE 12. SWELLING MAXIMA AND pH VALUES.

Acid.		Swelling Maximum.	pH of Solution.	pH of Gel.
		ccm.		
Hydrochloric . .	12° C.	41	2·4	2·9
Ditto . . .	20° C.	76	2·8	3·0
Nitric	12° C.	46	2·8	3·2
Ditto . . .	20° C.	78	2·4	2·5
Sulphuric . . .	12° C.	31	2·8	2·9
Ditto . . .	20° C.	47	2·9	2·4
Acetic . . .	12° C.	49	3·2	3·2
Chloracetic . . .	12° C.	50	2·8	3·1
Dichloracetic . .	12° C.	43	2·6	2·8
Trichloracetic . .	12° C.	50	2·9	3·0
a-Naphthalene-Sulphonic	12° C.	44	2·8	2·8
Pure water . . .	12° C.	14	—	—

The relation between the hydrogen ion concentration and the swelling of gelatin has also been studied in considerable detail by Ostwald, Kuhn and Böhme.[4] Examination of the curves for the swelling of three different gelatins after swelling for 72 hours in pure acids and in acid-salt mixtures, shows that for all the acids used there is a marked lowering of swelling as the pH value rises. Con-

[1] *Kolloid-Z.*, **50,** 130 (1930).
[2] *Kolloidchem. Beihefte*, **1,** 93 (1909–10).
[3] *J. Am. Leather Chem. Assocn.*, **15,** 228 (1920).
[4] *Kolloidchem. Beihefte*, **20,** 412 (1925).

siderable quantitative differences are exhibited by the gelatins. On the whole, a Meissner gelatin swells best although it is less pure. Considering the acids used, the order of diminishing effect is : acetic > salicylic > sulphosalicylic. Only in the region of minimum swelling do the curves intersect.

The fact that the higher ash Meissner gelatin swells more than an essentially pure gelatin contradicts Loeb's view that the salt content of a gelatin always reduces swelling.

Isoelectric gelatin gave the following characteristic acid series in relation to swelling : Hl > glycerophosphoric acid > sulphosalicylic acid > HCl > H_2SO_4. Especially noticeable is the fact that of the last two acids, H_2SO_4 at pH 2·7 promotes swelling better than does HCl. At higher hydrogen ion concentrations, HCl is the better for swelling.

Generalising, when gelatin swells in pure acids at the same pH value, swelling is different according to the acid employed. It cannot be admitted (as Loeb believed) that all acids of the same equivalency under the same conditions effect the same swelling. On the contrary, an accurate investigation of swelling by HCl over a narrow pH range showed that swelling is not as great as with the dibasic H_2SO_4, which, according to Loeb, should show only half the swelling effect.

On the whole, mixtures of acids and salts decrease swelling as compared with pure acids at similar pH values. Certain anions possess so strong a swelling effect that in buffer solutions containing them, gelatin may swell even more than in the pure acids of the same pH value. The isoelectric points of gelatins vary, and they vary too according to the buffer solutions in which they are dissolved. Thus, for Meissner and Stoess gelatins in the same buffer solution (sodium acetate + acetic acid) the isoelectric point corresponding to minimum swelling, is pH 4·7 and pH 5·2 respectively.

A very important investigation has recently been communicated by Miss Jordan-Lloyd,[1] who determined the swelling of pure gelatin [2] in the presence of different electrolytes over the temperature range 0°—25° C. Obvious advantages attach to working at 0° C., which is well below the transition point sol A $\rightleftharpoons$ gel B.

The results in brief showed that at 0° C., the swelling of gelatin in HCl is a maximum at pH 2·6. There is no solution of the gelatin in concentrated solutions of the acid. In the case of NaOH, minimum swelling is at pH 5 and as the pH rises to 7·5, the degree of swelling increases. Over the pH zone 7·5—10, swelling varies only slightly. At still higher pH values, a maximum swelling is noted at pH 11·5, whilst solution of the gelatin occurs at pH 13 and higher.

[1] *Biochem. J.*, **24,** 1460 (1930). See also Miss Pleass, *ibid.*, **24,** 1472 (1930).
[2] Jordan-Lloyd and Pleass, *ibid.*, **21,** 1352 (1927).

Over the temperature range investigated, the pH of maximum swelling is unaffected in acid solutions, but in the case of alkaline solutions the pH value of maximum swelling is higher the lower the temperature. At 25° C. solution of gelatin occurs in acid solutions of pH < 3 and without a maximum ; in alkaline solutions of pH $> 9{\cdot}5$, with maximum swelling at pH 7·2.

THE NATURE OF SWELLING

Various theories have been advanced concerning the swelling of gelatin. Fischer [1] considers that swelling and solution are two different processes, the former being hydration, the latter dispersion. He believes gelatin is capable of different degrees of polymerisation. At the higher temperatures, in presence of alkali or acid, the molecules are slightly associated ; the medium size molecules absorb most water and the large ones least. Hydration is increased by acids, the particles gaining the optimum size ; further acid reduces the size and tends towards solution.

Smith [2] advanced the theory that swelling is the result of osmotic pressure within the jelly, which functions as an imperfectly resisting membrane, the more so when highly swollen. Although osmotic pressure is the same at the optimum concentration of univalent acids and alkalies, the swelling in alkalies is much less inasmuch as the membrane effect is weakened.

Swelling has been considered [3] as a kind of solution, swelling being intensified with salts (except citrate) in proportion to the concentration of the salt. Acids exert a greater influence than salts, and HCl and H_2SO_4 of the same normality produce practically the same effect, maximum swelling being observed at N/60 (pH 1·7—1·8). The most effective anion is SCN, and citrate and tartrate are weakest.[4] The most effective cation is Ca. Mixtures of salts exhibit additive effects in the swelling phenomenon, the cations, however, retaining their individual characteristics. Whilst an acid added to a salt solution increases the swelling effect, a decrease is noted when a salt is added to the acid solution. The general conclusion is made that the hydrogen ion is most effective in promoting swelling, with calcium ion next, whilst the organic anions inhibit swelling.

Küntzel [5] distinguishes between two different influences of an electrolyte in the swelling of gelatin : (1) the adsorption of electrolyte by the gelatin, an " electrical " influence ; (2) peptisation. Although both influences are concurrent, the " electrical " pre-

[1] *Kolloid-Z.*, **17,** 1 (1915) ; *Science,* **42,** 223 (1915).
[2] *J. Am. Chem. Soc.*, **43,** 1350 (1921).
[3] von Moraczewski and Hamerski, *Biochem. Z.*, **208,** 299 (1929).
[4] *Vide* also Shreve, *J. Franklin Inst.*, **187,** 319 (1919).
[5] *Biochem. Z.*, **209,** 326 (1929).

dominates when gelatin swells in solutions of acids and bases, whilst when gelatin swells in salt solutions, peptisation predominates. Swelling is pronounced in acids or bases where one ion is adsorbed by the gelatin much more than the other ion, whereas the approximately equal adsorption of both ions (neutral salt effect) only produces slight swelling. Additive and antagonistic ion effects are discussed by Küntzel, who shows that gelatin may become isoelectric when the adsorption of anions and cations causes it to be charged equally positively and negatively ; the pH value alone does not define the isoelectric point. Peptisation *per se* does not produce swelling, but it augments the electrical swelling influence, different electrolytes possessing different peptising powers, the greater the molar solubility the greater being the peptising power.

Northrop and Kunitz[1] have measured the swelling pressure of solid gelatin in water and neutral salt solutions, finding it to increase rapidly between 15° C. and 37° C., the percentage change being nearly independent of the concentration of gelatin. They believe that gelatin consists of a network of insoluble fibres enmeshing a soluble constituent of gelatin, the osmotic pressure of which gives rise to the swelling pressure. Working on the theory that gelatin consists of a mixture of two or more substances, or group of substances, they isolated two fractions from gelatin by alcoholic precipitation at different temperatures. One fraction was soluble in cold water, formed no gel and had a low viscosity and a high osmotic pressure. The second fraction was insoluble in cold water, gave a gel in very low concentration and swelled much more than did ordinary gelatin (see p. 88 and p. 98).

The equilibrium conditions for the swelling of isoelectric gelatin in water have been discussed by Northrop,[2] and the kinetics of swelling by Northrop and Kunitz.[3] The diffusion of water into gelatin owing to the presence of soluble material (osmotic pressure) is opposed by the elasticity of the insoluble network and equilibrium is attained when the two pressures are equal. A slow secondary swelling can be observed, and this is attributed to the fatigue of the elastic force of the network. Whilst primary swelling is inversely proportional to the size of a block of gelatin, secondary swelling is independent of the size and is not regulated by the rate of diffusion of the water. Experimental data confirms the theoretical deductions that secondary swelling is proportional to the square root of the time and that with large blocks of gelatin at higher temperatures (up to 27° C.) swelling is entirely of the secondary type, the outer layers becoming fatigued and taking in more water before the inside layers undergo swelling.

[1] *J. Gen. Physiol.*, **10,** 161 (1926) ; *J. Phys. Chem.*, **35,** 162 (1931).
[2] *J. Gen. Physiol.*, **10,** 893 (1927).
[3] *Ibid.*, **10,** 905 (1927).

Knaggs [1] has shown that when a tissue swells in solutions of acids or alkalies, there is an intramolecular change in the protein. Working with collagen, such internal molecular change is particularly pronounced at pH 2 or less, and at pH 11 or more, *i.e.*, at and above the pH values for maximum swelling. "Between pH 3 and 10 the chief reaction which takes place is that the acid or alkali combines with the free amino—and carboxyl—groups, forming salts. At other concentrations free acid or alkali is present, which probably brings about intramolecular change." Evidence is presented in favour of two or more isomers of collagen, and the assumption made that the branched chains of gelatin form rings in the collagen molecule, such rings being ruptured during the extraction of gelatin with hot water. Gelatin swells more than collagen under similar conditions, and this is more marked at the point of maximum swelling. This is probably connected with the opening of the rings in the case of gelatin, providing additional points of attachment for acid or alkali and so permitting great increase in the hydration of the protein molecule.

Miss Jordan-Lloyd [2] has summarised the problem and believes "that no interpretation of the whole range of water absorption of gelatin is possible until further information is obtained on the influence of very dilute alkalies and of solutions of salts on the properties of water." She points out that there is now abundant evidence that three mechanisms of swelling are concerned in the gelatin-water system :—

(1) Imbibition, water being taken up to give the preformed structure ;

(2) osmotic swelling, characteristic of acid solutions and of alkaline solutions of pH $> 7{\cdot}0$. The unequal solvent pressure in the solution and in the jelly brings about such swelling ;

(3) Swelling due to hydration,[3] characteristic of salt solutions and of solutions with pH between $5{\cdot}5$ and $7{\cdot}0$.

THE SURFACE TENSION OF GELATIN SOLUTIONS

Different investigators have reported that the surface tension of gelatin solutions reaches a minimum value at the isoelectric point (usually given as pH $4{\cdot}7$) with maximum values in the acid and in the alkaline regions. Table 13 collects several such observations and the interesting discrepancies thus made evident receive explanation later.

[1] *Biochem. J.*, **23,** 1308 (1929).
[2] *Ibid.*, **24,** 1470 (1930).
[3] Hydration swelling is considered in detail by Neville and Theis, *Am. Coll. Symp. Monog.*, **7,** 41 (1930), and *Ind. Eng. Chem.*, **22,** 57 (1930). See also Northrop and Kunitz, *J. Phys. Chem.*, **35,** 162 (1931), and Kunitz, *J. Gen. Physiol.*, **10,** 811 (1927).

TABLE 13. SURFACE TENSION OF GELATIN SOLUTIONS AS A FUNCTION OF pH VALUE.

Maxima.	Minima.	Observer.
—	4·7	Jol in.[1]
—	4·7	Shukoff and Bushmakin.[2]
2·85 8·30	4·7	Jermolenko.[3]
4·7 2·8—3·0	3·8—4·0 9·0	St. Johnston and Peard.[4]
3·0—3·2 8·0—9·0	4·5—4·7	Bottazzi.[5]
8·0—9·0 3·0	4·5	de Caro.[6]

Bottazzi holds that for the maximum lowering of surface tension the gelatin must be present as undissociated molecules, a conclusion shared by de Caro and by Artom.[7] The latter author made a detailed investigation on the surface tension of gelatin solutions, interpreting his data on considerations of the change in molecular aggregation under the different conditions applying. Thus, using 1 per cent. solutions of isoelectric gelatin, the surface tension decreases over the temperature range 20°—38° C., since increasing temperature transforms the surface-inactive molecular aggregates into the strongly surface-active free molecules.

He found that an isoelectric gelatin in solutions at different pH values showed a minimum surface tension at the isoelectric point and maximum values at pH 2·6 and pH 10·0. Such variations are explained on the theory that the gelatin ions do not lower surface tension so effectively as the gelatin molecules. Ageing the 1 per cent. solutions resulted in a gradual lowering of surface tension, interpreted as due to the formation of intermediate hydrolytic products.

If to gelatin solutions at the pH values of maxima surface tensions there be added equivalent quantities of acids, alkalies or neutral salts, the surface tension is diminished to an extent depending on the nature and concentration of the anion and cation added, the reduction being the greater the more pronounced the affinity of the ion for the solvent.

[1] *J. Phys. Chem.*, **29,** 271 (1925).
[2] *J. Russ. Phys.-Chem. Soc.*, **59,** 1061 (1927).
[3] *Kolloid-Z.*, **48,** 141 (1929).
[4] *Biochem. J.*, **19,** 281 (1925).
[5] Alexander's "Colloid Chemistry" (New York, 1928), Vol. 2, p. 150.
[6] *Atti. accad. Lincei* (6), **1,** 729 (1925); de Caro and Laporta, *Arch. sci. biol. Italy*, **14,** 264 (1930).
[7] *Boll. soc. biol. sper.*, **4,** 605 (1929); *Arch. sci. biol. Italy*, **14,** 327 (1930).

The possibility that there might be a change in the surface tension of a gelatin sol after being set to a gel and then restored to the original temperature has been examined by Pupilli.[1] Surface tension measurements were made over the pH range 2—10. The sols were congealed at −12° C. to −18° C. for 30—45 minutes, and then reliquefied at room temperature. The surface tension of the congealed and reliquefied sols is greater than the original surface tension for those sols in the isoelectric range (around pH 4·7). It is unchanged over the pH ranges 3—3·4 and 8—9 ; beyond these zones it is increased. Such surface tension changes are considered due to the formation of molecular aggregates and to the varying degrees of hydration of the gelatin. The diminution of the attractive forces between gelatin and water is not confined to the isoelectric zone ; both on the acid and the alkaline side hydration of the dispersed phase increases and may pass through a maximum, such hydration, however, being progressively decreased as electrolytes are added.

An extended investigation by Davis, Salisbury and Harvey [2] concerns the variation in surface tension of gelatin sols with concentration, temperature, pH value, and ageing. Working at pH 4·2 and pH 8, at temperatures 25° C. and 40° C., it was found that the surface tension falls rapidly up to about 1 per cent. gelatin and then more lowly until the gel condition interferes with the determinations. The influence of temperature was studied with 1 per cent. and 3 per cent. sols at pH 8. The 1 per cent. sol exhibited a rapid fall in surface tension as the temperature was raised to 38° C., when a sudden change in the graph indicated a constant value. The 3 per cent. sol, however, showed a maximum surface tension at about 28° C., and at 38° C., the same sudden change to a constant value. The authors believe 38° C. to be a transition point as mentioned by Davis and Oakes [3] (see p. 96).

As regards the influence of pH, isoelectric hide gelatin was prepared by Loeb's method.[4] This ash-free gelatin did not lower the surface tension to the same extent as the original gelatin. At 25° C., the surface tension had a maximum value at about pH 3·5 and a minimum at pH 9·5 (0·5 per cent. sol) and pH 8·9 (1 per cent. sol). The original gelatin in 1 per cent. solution showed the same maximum, but the minimum value was at pH 5·8.

Recent work by Johlin [5] has shown that due to the surface concentration of the gelatin, the results of surface tension measurements made after the system has attained equilibrium (24 hours), are entirely different to those obtained in 1 to 3 hours after formation

[1] *Arch. sci. biol. Italy*, **12,** 373 (1928).
[2] *Ind. Eng. Chem.*, **16,** 161 (1924).
[3] *J. Am. Chem. Soc.*, **44,** 464 (1922).
[4] *Ibid.*, **44,** 213 (1922).
[5] *J. Biol. Chem.*, **87,** 319 (1930).

of the air/liquid interface. A static method of measuring surface tension is absolutely necessary.

One per cent. solutions of ash-free gelatin were examined at 37° C., using the sessile bubble method.[1] over a pH range 1—12. A maximum value was observed at pH 9. The graph of the *equilibrium* values of surface tension does not show the usual minimum previously observed at the isoelectric point or the maximum on the acid side of this, although the corresponding curves for surface tension at intervals of 1, 2 and 3 hours do. Johlin sug-

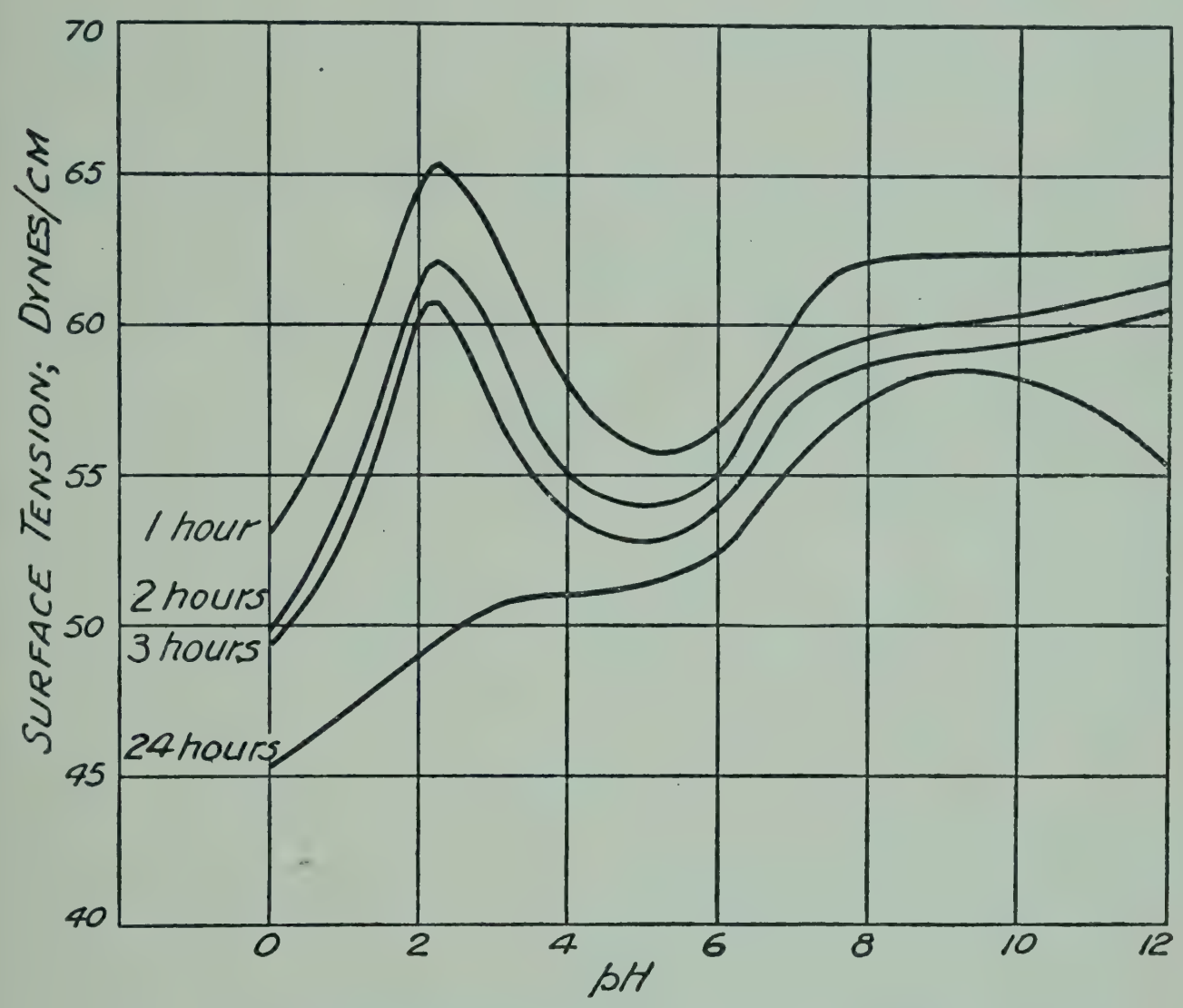


FIG. 7.—The surface tension of 1 per cent. ash-free gelatin solutions at 37° C. in relation to pH value.

gests that possibly acid hydrolysis is responsible for the deviation. Fig. 7 by Johlin, illustrates these surface tension changes.

THE VISCOSITY OF GELATIN SOLUTIONS

The viscosity of a liquid is its internal friction, *i.e.*, its resistance to shear, agitation or flow. Viscosity is measurable in absolute units, viz., the poise, which is defined [2] as the force required to produce a difference in the velocity of flow of a liquid of 1 cm. per second, when this force is exerted on 1 sq. cm. between two parallel planes, each 1 sq. cm. in area and 1 cm. apart. The reciprocal of absolute viscosity is the fluidity. Viscosities are frequently

[1] Johlin, *J. Phys. Chem.*, **29,** 1133 (1925).
[2] Deely and Parr, *Phil. Mag.*, **26,** 87 (1913).

expressed in centipoises, *i.e.*, in $\frac{\text{poise}}{100}$, and the standard for usual reference is the viscosity of water at 20° C., viz., 1·0050 centipoise.

There is a distinction necessary between viscosity and plasticity ; in the former, the ratio : $\frac{\text{shearing stress}}{\text{rate of shear}}$ is a constant, whilst plasticity is known by the fact that this ratio varies with the rate of shear.[1] A plastic system requires the expenditure of energy to cause it to " yield " before plastic flow commences, and hydrophile colloids such as gelatin hydrosols, are of this type and are characterised by viscosity values much greater than that of the pure solvent.

Various methods are employed for measuring viscosity, but their relative merits and the theoretical foundation of their use are beyond the scope of this volume.[2] The various methods though giving comparable results for pure liquids and true solutions yield discordant data in the case of hydrophile sols due to the plastic nature of the colloids set up by a definite structure in solution, such " structural viscosity " being particularly evident with a sol capable of undergoing gelation.[3]

The most commonly employed instrument has been the Ostwald viscometer in which a head of the liquid produces flow through a capillary tube. Poiseuille's equation for the viscosity determined in this way is :—

$$\eta = \frac{\pi r^4 pt}{8lv}$$

where η = viscosity.
r = radius of capillary tube,
l = length of capillary tube.
v = volume of liquid flowing under pressure, p.
t = time of efflux of liquid.

According to Hatschek,[4] the marked variableness of viscosity with rate of shear is not directly exhibited with the Ostwald instrument and " the viscosity-concentration or viscosity-temperature curves are still in general smooth, although to every determination corresponds a different velocity and therefore a different rate of shear. In addition to this, there is in most cases a critical velocity above which the viscosity appears to be independent

[1] See Herschel, Alexander's " Colloid Chemistry " (New York, 1926), Vol. 1, p. 727 ; Hatschek, *ibid.*, p. 738 ; Ostwald, " Handbook of Colloid Chemistry " (London, 1919), p. 145 ; Bingham, " Fluidity and Plasticity " (New York, 1922), and in Alexander's " Colloid Chemistry," Vol. 1, p. 720.

[2] Hatschek's " Viscosity of Liquids " (London, 1928) gives full details.

[3] Hatschek, *Kolloid-Z.*, **13,** 88 (1913) ; Hess, *ibid.*, **27,** 154 (1920) ; Rothlin, *Biochem. Z.*, **98,** 34 (1919).

[4] Alexander's " Colloid Chemistry," Vol. 1, p. 744. See criticism by Freundlich, " Colloid and Capillary Chemistry " (London, 1926), p. 541, last paragraph.

of the rate of shear and this critical velocity is probably exceeded in many cases, especially with narrow capillaries and moderate times of efflux."

The viscosity of gelatin sols has been studied extensively in attempts to throw light on the nature of the sol, the effect of age, the acid and alkali protein equilibrium, and the sol $\rightleftharpoons$ gel equilibrium. The ash content of the gelatin appears to have a profound influence on the viscosity, high ash content being correlated with abnormally low viscosities.[1]

VISCOSITY AS A FUNCTION OF CONCENTRATION

Any attempt at mathematical formulation of the correspondence between the concentration and viscosity of gelatin seems out of the question, since at low temperatures gelation will be encountered and at high temperatures hydrolysis is taking place.

In dilute solutions there is a linear relation between viscosity and concentration, but not in more concentrated solutions. Thus, Levites [2] gives the following observed values at 35° C. referable to water as unity at the same temperature :—

Gelatin Concentration.	Viscosity.
0·25 per cent.	1·10
0·50 ,, ...	1·22
0·75 ,, ...	1·32
1·00 ,, ...	1·46
1·50 ,, ...	1·75
2·00 ,, ...	2·05
3·00 ,, ...	2·96

At low temperatures, the rise in viscosity with increasing concentration is most marked. Thus, at 0·5° C., a 0·1 per cent. solution had a viscosity of 2·1 centipoise, whereas a 1 per cent. solution gave the value 2,243 centipoise.[3]

Recently it has been shown [4] in measurements of the viscosities of dilute ash-free gelatin solutions at 20°, 25° and 35° C., that there is a step in the concentration-viscosity curve at about 0·1 per cent. concentration, at 20° and 25° C., whereas at 35° C. the concentration-viscosity graph is continuous. This step is observed only when the solution is more acidic than the isoelectric point of the gelatin and is explained on the Smith theory of sol $\rightleftharpoons$ gel equilibrium (see p. 96).

[1] Oakes and Davis, *Ind. Eng. Chem.*, **14,** 708 (1922); Loeb, *J. Gen. Physiol.*, **3,** 391 (1921).
[2] *Kolloid-Z.*, **2,** 210 (1907).
[3] Dahlberg, Carpenter and Hening, *Ind. Eng. Chem.*, **20,** 520 (1928).
[4] Fuseya, Sasaki and Nagano, *J. Phys. Chem.*, **34,** 2012 (1930).

VISCOSITY AS A FUNCTION OF THERMAL HISTORY

Gelatin solutions are usually prepared by soaking the gelatin in water for some time and then either heating or adding hot water. The time of soaking above 1 hour in water at 25° C. has no influence on viscosity.[1] Whilst Manning [2] believes that the heating of a gelatin solution, even for a short time, has a profound influence on the subsequent viscosity measurements, Davis and Salisbury [3] demonstrated that the usual thermal conditions employed in preparing gelatin solutions are entirely without influence on viscosity. However, Sheppard and Houck [4] find that the time of any preheating above 40° C. should be as short as possible, and that both time and temperature of the preheat must be constant for comparable data.

Prolonged heating has a pronounced effect on viscosity, as the following data by von Schroeder [5] illustrate :—

Time Heated at 100° C.	Viscosity (Water = 1).		
(Hours).	1 per cent. Sol.	2 per cent. Sol.	3 per cent. Sol.
0·5	1·29	1·75	—
1·0	1·23	1·55	—
1·5	1·20	1·49	—
2·0	1·17	1·47	1·76
2·5	1·15	—	—
3·0	1·14	1·37	1·68
4·5	1·1	—	—
5·0	—	1·30	1·54
8·0	—	1·25	1·47
12·0	—	1·23	1·40
16·0	—	1·22	1·39

In such treatment hydrolysis takes place, at a rate in accordance with the equation :—

$$-\frac{d\eta}{dt} = \mathrm{K}(\eta - \eta_e)$$

where η = viscosity at time t.
η_e = lowest value of viscosity.
K = constant.

Rawling [6] has confirmed von Schroeder's results.

[1] Sheppard and Houck, *ibid.*, **34,** 274 (1930).
[2] *Biochem. J.*, **18,** 1085 (1924).
[3] *Kolloid-Z.*, **49,** 274 (1929).
[4] *J. Phys. Chem.*, **34,** 275 (1930).
[5] *Z. physik. Chem.*, **45,** 75 (1903). McNally and Vanselow, *J. Am. Chem. Soc.*, **52,** 3846 (1930), show that the fluorescence of gelatin in ultraviolet light decreases as the aggregates responsible for the high viscosity of the sol are broken up by boiling.
[6] *Vide* Slater Price, Adhesives Research Comm., *Rept.*, **1,** 76 (London, 1922).

VISCOSITY AS A FUNCTION OF THE AGE OF SOLUTIONS

That the viscosity of a gelatin solution increases with age has been generally accepted without qualification. Von Schroeder and Levites early presented data supporting this, *e.g.*, the former gives the following data :—

TABLE 14.

Influence of Age on Viscosity of Gelatin Solutions.

Time Elapsing.	Viscosity at Temperature.		
(Mins.).	21° C.	24° C.	31° C.
5	1·83	1·65	1·41
10	2·10	1·69	1·41
15	2·45	1·74	1·42
30	4·13	1·80	1·42
60	13·76	1·90	1·42

It is evident that the viscosity increase is marked at 21° C. and practically undergoes no change at 31° C. Obviously, some secondary change is setting in at the lower temperature. Levites obtained similar data and believed that for any given gelatin solution there is a certain minimum temperature where viscosity remains a constant.

According to Arisz [1], who worked with solutions of gelatin in glycerol-water, 65° C. represents a critical temperature above which an irreversible change (hydrolysis) to β-gelatin occurs, and the viscosity remains constant after 24 hours. Below 65° C. an equilibrium is reached, whilst below 44° C. the attainment of equilibrium is very slow and gradual setting to gel takes place. Taking 65° C. as the critical temperature for equilibrium attainment, it was found that rapid cooling of a sol to below 65° C. resulted in a solution of which the viscosity increased with time, whilst a solution at (say) 46° C. which is heated quickly to 55° C. shows a viscosity which decreases with time. Another interesting observation due to Arisz,[2] is that a 10 per cent. gelatin solution in glycerol-water at 50° C. shows decreasing viscosity on repeated passage through the Ostwald viscometer, whereas at 65° C. the viscosity is constant.

Modern work has extended these observations. Davis and Oakes [3] found that the ageing of gelatin solutions at and below 35° C. resulted in increasing viscosity, whilst the viscosity is unchanged when ageing takes place above 40° C. Interesting results were obtained by Nagorny,[4] employing gelatin solutions

[1] *Kolloidchem Beihefte*, **7**, 1 (1915).
[2] Cf. Gokun, *Kolloid-Z.*, **3**, 84 (1908).
[3] *J. Am. Chem. Soc.*, **44**, 464 (1922).
[4] *Kolloid-Z.*, **41**, 123 (1927).

sterilised by boiling and then incubated for about 49 days at 6° C. and 37° C. respectively, the viscosity being measured at intervals. Measuring the viscosity at 20° C. the gelatin incubated at 6° C. showed an increasing viscosity with age, whilst the solution incubated at 37° C. showed a decreasing viscosity. However, if the viscosity measurements are made at 30° C. the viscosity in each instance remains practically constant. At 20° C. the 37° C. sample approximately obeyed Poiseuille's rule, and at 30° C. both samples did so. The 6° C. sample could be fractionated by $(NH_4)_2SO_4$ to give two fractions, one of which gelatinises at ordinary temperature.

Nagorny advances the theory that in the 6° C. sample, the particles are larger, their size remaining unchanged at 20° C., but undergoing dispersion at 30° C. Although the difference between the small and the large particles may be merely one of dimensions,[1] the experiments on fractional precipitation with $(NH_4)_2SO_4$ support the view that there is a chemical difference (see p. 79).

VISCOSITY AS A FUNCTION OF THE pH VALUE

Von Schroeder observed that in HCl and NaOH gelatin had a maximum viscosity, corresponding to N/256 HCl and N/28 NaOH. A mass of data has since accumulated on this problem, chiefly from Loeb's laboratory, where viscosity is considered in relation to the pH value of the solution.

The following table summarises some of the more important published results :—

TABLE 15.

Viscosity of Gelatin Solutions.

Maximum Value.	Minimum Value.	Observer.
pH 3 and pH 9	pH 4·9	Bogue.[2]
pH 3·2	pH 4·7	Loebel.[3]
pH 2·5 and pH 9·5	—	Shukoff and Bushmakin.[4]
pH 2·9—3·0	—	Shito.[5]
—	pH 4·7	Hitchcock.[6]
pH 2·75 and pH 7·4	pH 5·0 and pH <1	Freundlich and Neukircher.[7]
pH 3·0—3·5	—	Davis, Oakes and Browne.[8]
pH 3·0	—	Oakes and Davis.[9]
pH 2·5—2·6	pH 5·5 ; pH 4·7 (different gelatins)	Davis and Salisbury.[10]

[1] Cf. Fikentscher and Mark, *Kolloid-Z.*, **49,** 135 (1929), who discuss the influence of molecular size (or chain length) of lyophilic colloids on viscosity.

[2] *J. Am. Chem. Soc.*, **44,** 1343 (1922).

[3] *J. Phys. Chem.*, **32,** 763 (1928).

[4] *J. Russ. Phys.-Chem. Soc.*, **59,** 1061 (1927).

[5] *Hokkaido J. Med.*, **7,** 187 (1929).

[6] *J. Gen. Physiol.*, **6,** 457 (1924).

[7] *Kolloid-Z.*, **38,** 180 (1926).

[8] *J. Am. Chem. Soc.*, **43,** 1526 (1921).

[9] *Ind. Eng. Chem.*, **14,** 706 (1922).

[10] *Ibid.*, **20,** 829 (1928).

Davis and Salisbury [1] employed 1 per cent. gelatin solutions (on dry basis) to determine the influence of the prehistory of the gelatin on viscosity, examining over the pH range 1 to 9 and using pigskin and ossein gelatins, as well as a special high-grade gelatin of unknown origin. Fig. 8 reproduces two of the authors' graphs : (A) is an acid-boiled pigskin gelatin, (B) is a high-grade gelatin. The remarkable difference in the viscosity curves is clearly brought out.

(A) shows a viscosity maximum at pH 2·5—2·6, the curve

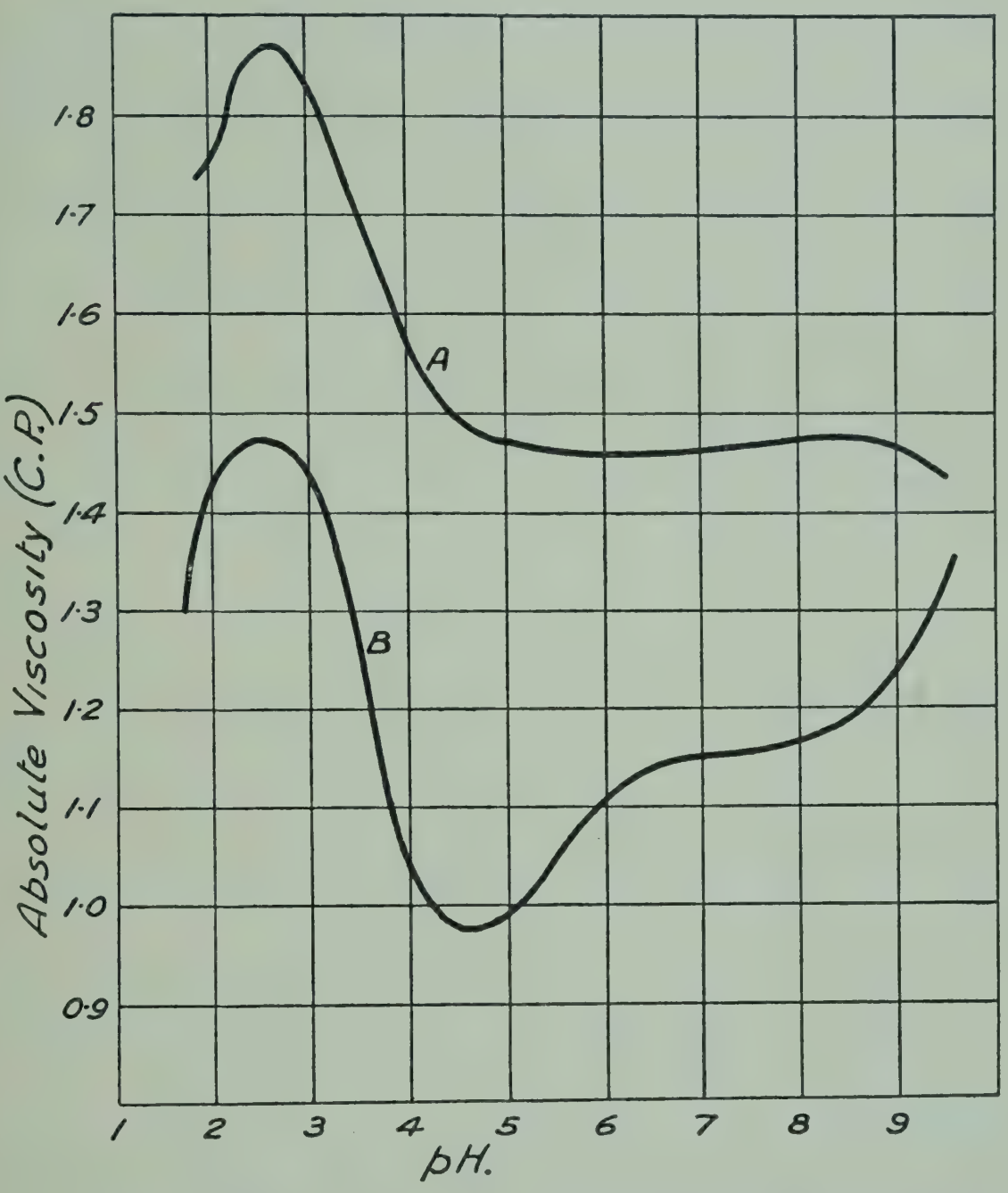


FIG. 8.—Viscosity-pH curves of gelatins of different histories.

flattening out when reaching pH 5·5. (B) shows a viscosity maximum at pH 2·5—2·6 and a minimum at pH 4·7 ; thereafter, up to pH 9·4, the viscosity rises to a maximum almost equal to that at pH 2·6.

The unexpected and undesirable results occasionally found in the factory when gelatin preparations fail to set are undoubtedly sometimes to be attributed to a change in the biological origin and/or the chemical derivation of the gelatin in question. Jelly-strength is intimately connected with the type of viscosity curve,

[1] *Kolloid-Z.*, **49**, 270 (1929).

gelatins of low jelly-strength, giving flattened-out curves for the viscosity-pH relation.

The shape of the viscosity-pH curve is greatly influenced by the age of the gelatin solution, as shown by Sheppard and Houck.[1] Fig. 9 illustrates this point, and is reproduced from the authors' publication. It is obvious that in comparing the viscosities of

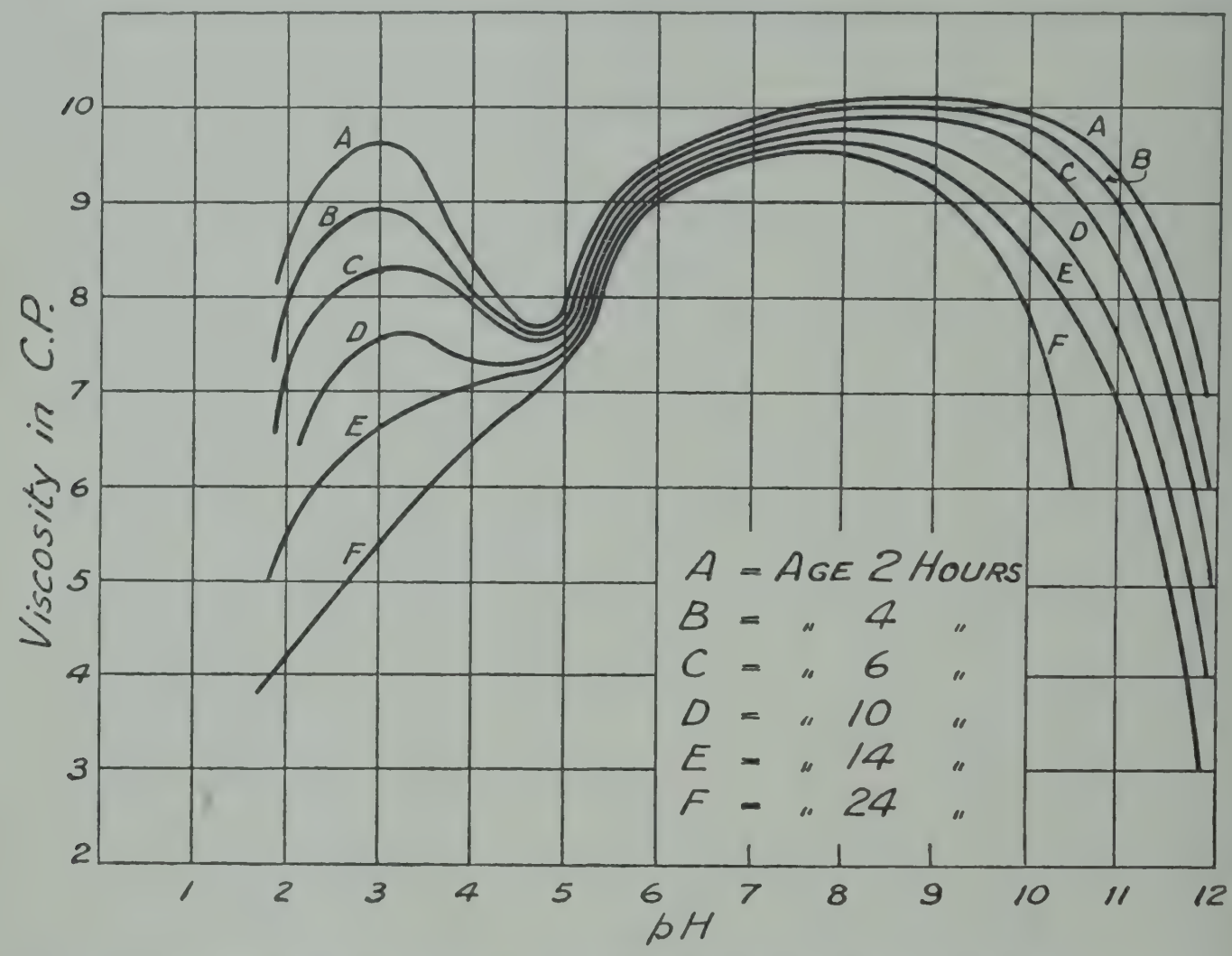


Fig. 9.—The viscosity-pH curve of de-ashed gelatin as influenced by the age of the solution.

different samples of gelatin, it is necessary to ensure constant factors for concentration, thermal history, pH value and age.[2]

VISCOSITY AS A FUNCTION OF ELECTROLYTES

Sen [3] has discussed the general problem of the viscosity of colloidal solutions in the presence of electrolytes. The viscosity of the sol will increase as the water envelope on the dispersed particles becomes thicker. Electrolytes at first increase the potential of the electrical double layer at the surface of the particles, but further electrolyte decreases the charge owing to coagulation. The former effect increases the viscosity and the latter decreases it, so that a minimum would be anticipated in the viscosity-electrolyte concentration curve. Again, the proteins contain particles which "bind" water so that this no longer acts as a solvent and the

[1] *J. Phys. Chem.*, **34,** 273 (1930).
[2] See Briefer and Cohen, *Ind. Eng. Chem.*, **19,** 252 (1927).
[3] *Kolloid-Z.*, **38,** 253 (1926).

viscosity is, in consequence, high.[1] In the presence of acid or alkali, solvation of the gelatin particles is not the primary cause of increased viscosity but rather the formation of adsorption compounds with the acid or alkali.[2]

Extensive experiments on the influence of electrolytes on the viscosity of colloids have led Dhar and his co-workers [3] to the fundamental conception that viscosity and hydration of a sol are greater the smaller its electric charge. Adsorption of ions lowers the viscosity, since the surface of the colloid particle is less when its charge increases. Dhar employed 1 per cent. gelatin solutions at 30° C., in the presence of NaOH, HCl, KCl and $BaCl_2$. In the presence of small amounts of these electrolytes, there is a slight fall in viscosity which is connected with the increase in electric charge. At higher electrolyte concentrations the viscosity rises, continuously with KCl and $BaCl_2$, but to a maximum and then further decreases with NaOH and HCl. In his opinion, KCl and $BaCl_2$ in increasing concentration reduce hydration and, therefore, viscosity. At very high concentrations of NaOH and HCl the gelatin probably forms a molecularly dispersed solution [4] and, therefore, the volume of the dispersed portion is smaller with consequent decrease in viscosity.

VISCOSITY AS A FUNCTION OF ORIGIN

It is agreed that the nature of the precursor of a gelatin, and also the chemical treatment given in extracting the gelatin, profoundly influence the physical properties. Kernot and Knaggs [5] give the following table illustrating this point in connection with the viscosity of gelatin.

TABLE 16. RELATIVE VISCOSITY OF GELATIN SOLUTION AT 40° C.

Gelatin Conc. Per cent.	Ossein.		Horn-pith.		Isinglass.	Calf-skin.		Fish-skin.	
	Acid.	Alkali.	Acid.	Alkali.		Acid.	Alkali.	Acid.	Alkali.
5·0	2·584	4·147	2·702	6·040	6·662	3·278	4·477	4·477	7·158
1·25	1·222	1·420	1·317	1·582	1·490	1·371	1·421	1·591	1·651
0·5	1·085	1·200	1·133	1·169	1·194	1·142	1·220	1·285	1·294
0·05	1·041	1·037	1·059	1·002	1·028	1·014	1·037	1·085	1·046
0·005	1·019	1·019	1·007	—	1·010	—	1·016	0·993	1·014

[1] Cf. Freundlich, *Ber.*, **61 B,** 2219 (1928).
[2] Fodor and Mayer, *Kolloid-Z.*, **44,** 314 (1928).
[3] *Ibid.*, **44,** 225 (1928).
[4] But see Lewis and Daniel, *Am. Coll. Symp. Monog.*, **4,** 130 (1926).
[5] *J. Soc. Chem. Ind.*, **47,** 98 T (1928).

THE TURBIDITY OF GELATIN SOLUTIONS

The question of the turbidity of gelatin solutions is important to the food chemist, whose aim is to produce brilliantly clear bottled jellies and table jellies (prepared from "jelly tablets" or from powdered gelatin, the so-called "jelly crystals").

Apart from the turbidity which may arise from ash impurities, such as calcium salts (and the nature of the water used in preparing the gelatin products) there is a turbidity due to the colloidal behaviour of the gelatin itself in aqueous solution, and is much the more important factor to be considered.

Early workers, like Arisz [1] and Dhéré and Gorgolewski,[2] pointed out that gelatin solutions became turbid below 30° C., becoming clear again at $> 30°$ C., the effect *increasing* with *decreasing* ash content. Traces of acid or alkali were effective in removing such turbidity. They also observed that the turbidity increased to a maximum and then decreased as the concentration of gelatin increased, maximum turbidity occurring at about 2·5 per cent. gelatin.

Modern work [3] identifies maximum turbidity of gelatin with its isoelectric point. Many investigators have studied the relation of the Tyndall effect in gelatin solutions as a function of pH and temperature in an endeavour to elucidate the condition of gelatin in the sol form. Thus, Kraemer and Dexter,[4] employing 1 per cent. solutions of gelatin, found that at 40°—50° C., where gelatin is in the sol form, there is little light-scattering capacity and this almost independent of the temperature and the pH value of the solution. About pH 5 there occurs a relatively slight rise in the intensity of turbidity. On lowering the temperature so that the gel form appears, an extremely pronounced turbidity appeared at the isoelectric point, pH 4·95. Examining the range pH 2·5 to 12, the authors found that the increase in Tyndall effect was limited to the zone pH 4·5 to 7·5. At pH $< 4·5$ and $> 7·5$, decreasing the temperature from 50° C. to 17° C. had practically no influence on the Tyndall effect. At 17° C. the pH influence is important, *e.g.*, there is at least a 400 per cent. increase in the Tyndall effect for a pH variation of 0·03 (pH 4·87 to pH 4·90).[5]

Although the relation of Tyndall intensity to pH does not parallel the combination curve for gelatin, there is some corre-

[1] *Proc. Acad. Sci., Amsterdam,* **26,** 331 (1913); *Kolloidchem. Beihefte,* **7,** 22 (1915).

[2] *J. physiol. path. gén.*, **12,** 645 (1910); **13,** 157, 167 (1911).

[3] Cf. Kraemer and Fanselow, *J. Phys. Chem.*, **29,** 1169 (1925); Vlès and Vellinger, *Compt. rend.*, **181,** 1189 (1925); Sheppard and Houck, *J. Phys. Chem.*, **34,** 2189 (1930).

[4] *J. Phys. Chem.*, **31,** 764 (1927).

[5] Kraemer, *Am. Coll. Symp. Monog.*, **4,** 102 (1926).

spondence with the rapid decrease in the case of flocculation by alcohol as the pH decreases from the isoelectric point [1] (see p. 49).

Kraemer and Dexter argue that at temperatures below 30° C., gelatin sols contain solution units or micelles, which are well-defined and highly dispersed. Equilibria changes arising from variation in pH or temperature are rapid, *i.e.*, there is no hysteresis. On decreasing the temperature turbidity appears due to the increase in size of the gelatin micelles.[2] Traces of acid or alkali repress this turbidity, but the mechanism (resembling peptisation) is obscure.

Somewhat different results have been reported by Mayer,[3] who subjected his gelatin to electrodialysis. The pure gelatin in 1 per cent. solution had a pH value, 4·8—4·9, and exhibited marked turbidity at lower temperatures. Addition of acid or alkali produced definite maxima and minima turbidities, viz., maxima at pH 5·4—5·6 and pH 4·0—4·2 ; clear solutions obtained at pH < 3 and pH 6—7. Working at 12·5° C., 17·5° C., 22·5° C., and 27·5° C., and with different gelatin concentrations up to 4 per cent., maximum turbidity at all these temperatures occurred at a gelatin concentration of 1·8 per cent., the greatest absolute turbidity being at the lowest temperature. Mayer believes the explanation of the phenomena lies in the alteration in degree of dispersion of gelatin [4] with temperature or in the degree of hydration in the presence of acid or alkali.[5]

The influence of neutral salts in regaining clarity in turbid gelatin solutions was investigated by Gerngross,[6] who added 2 c.c. 0·1 N. salt solutions to 15 c.c. of 1 per cent. gelatin solution. At 18° C. his 1 per cent. gelatin solution exhibited maximum turbidity at pH 5·0. The order of efficiency of the salts employed in regaining clarity was : $K_2SO_4 > KCNS > KI > KBr > KNO_3 > KCl$.

Occasionally in technical operations the unexpected turbidity of gelatin solutions is disturbing and apparently without explanation. Recent important observations by Briefer [7] have, however, cleared up this problem and shown the remarkable influence of the biological precursor of gelatin and its treatment.

Careful quantitative measurements of the turbidity of solutions of four different gelatins were made with varying pH factor. The four types of gelatin were extracted from : (A) acid-plumped pig-skins, (B) limed splits, (C) limed sinews, (D) limed pieces. Fig. 10,

[1] Cf. Loeb, *J. Gen. Physiol.*, **3,** 257 (1919).

[2] See Backmann, *Z. anorg. allgem. Chem.*, **73,** 125 (1912), who studied maximum turbidity in gelatin sols of 1–2 per cent. concentration by the ultramicroscope.

[3] *Kolloid-Z.*, **44,** 315 (1928).

[4] Supported by the experimental data of Stiasny *et al.*, *Collegium* (**1925**), p. 23, who announced a similar idea.

[5] Cf. the views of Wo. Ostwald, " Licht und Farbe in Kolloiden " (Dresden and Leipzig, 1924), p. 33.

[6] *Kolloid-Z.*, **40,** 279 (1926).

[7] Briefer and Cohen, *Ind. Eng. Chem.*, **20,** 408 (1928) ; Briefer, *ibid.*, **21,** 266 (1929).

reproduced from Briefer and Cohen's paper, illustrates the pronounced difference in the positions of turbidity maxima, gelatin A being in the region pH 8, and the gelatins from limed stock in the region pH 4.9 Fig. 11 reproduces the authors' photographs of the phenomenon.[1]

Briefer's opinion is that "the turbidity curve is an almost unfailing index of the approximate position of the isoelectric point." All gelatin raw stock (bone, pigskin, calfskin, sinews, etc.) if

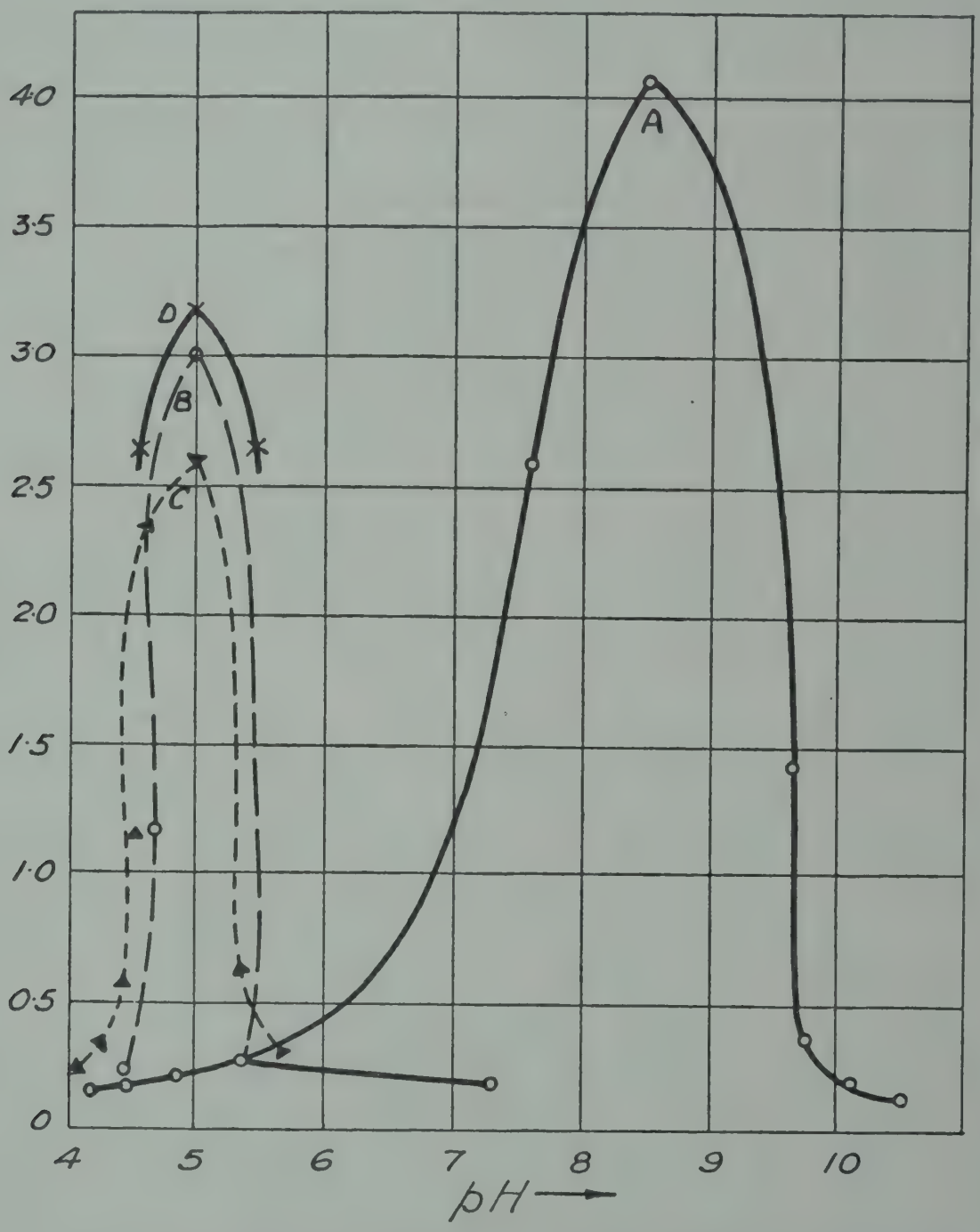


FIG. 10.—Turbidity-pH curves of various gelatins (ordinates express turbidity densities).

originally conditioned with lime, furnishes gelatin which exhibits maximum turbidity on the acid side of the pH scale. This holds good even if the lime is washed out of the material or, indeed, removed by acid. If, however, the same raw materials are originally acid-conditioned, the gelatins have isoelectric points in the region pH 7—8, with consequent maximum turbidity (see p. 67). So characteristic is such behaviour that it is uninfluenced by subsequent de-ashing.

It was noted that the turbidity effect varies in intensity in

[1] Cf. photographs published by Mayer, *Kolloid-Z.*, **44,** 318 (1928), and by Ostwald, *loc. cit.*, p. 30.

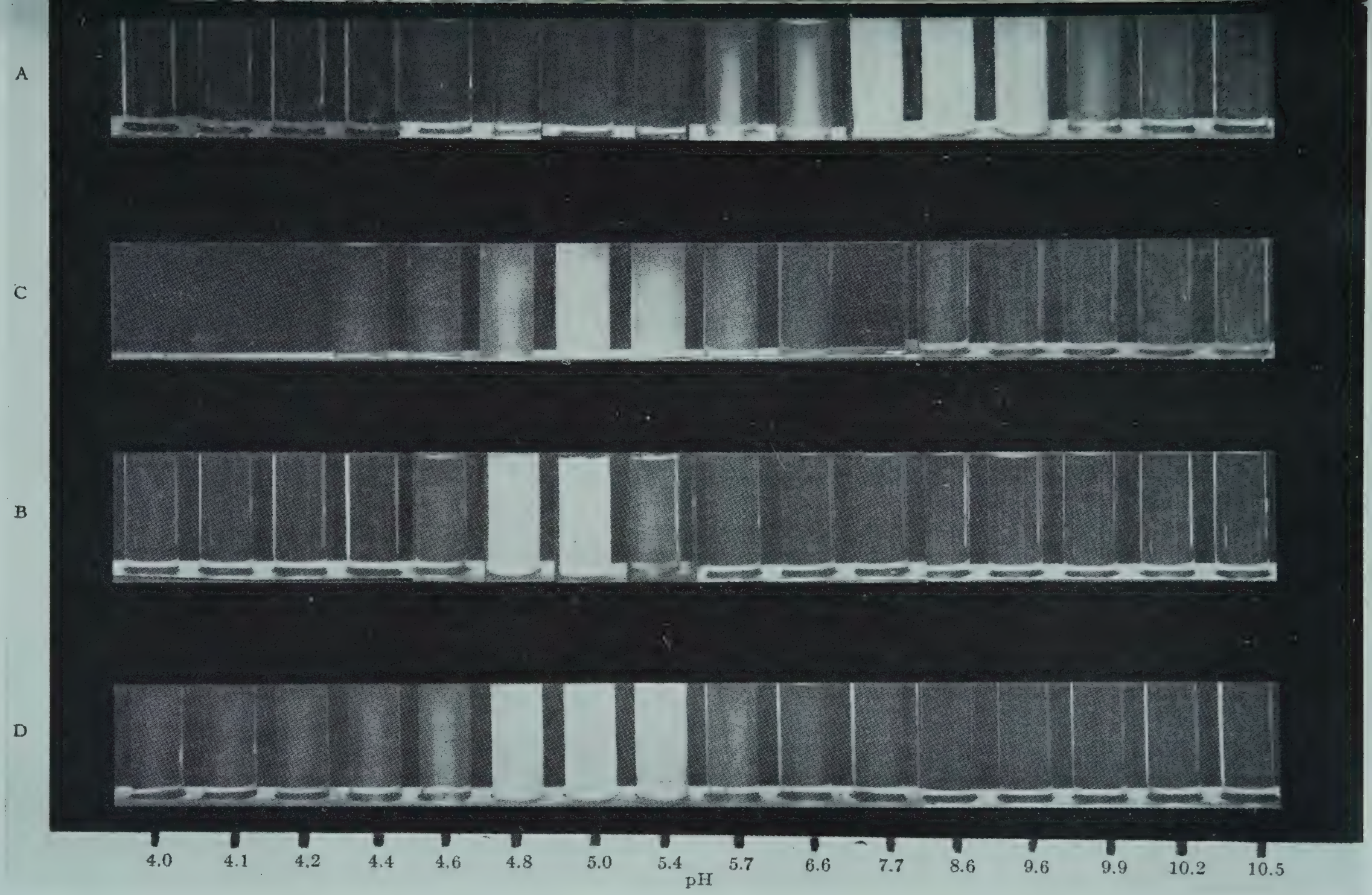


FIG. 11.—Photographs of turbid gelatin jellies at various pH values.

[*To face p.* 94.

different gelatins, and "it seems obvious that the intensity of turbidity is related to the degree of hydration resulting from manufacturing procedure." The turbidity increases with lowering of temperature and is reversible. In due time the turbid matter coagulates and settles out. As regards the influence of gelatin concentration on turbidity, a maximum effect is observed in the region of a 1 per cent. solution of isoelectric gelatin, although about 1·5 per cent. concentration is necessary in the case of low-grade samples.

An important communication from the laboratory of Jordan-Lloyd has been presented by Miss Pleass,[1] who examined the turbidity of gelatin gels over a concentration range of 0·1 per cent. to 15 per cent., at temperatures of 0°, 12°, 18° and 25° C. Maximum turbidity was exhibited in gels containing 2 per cent. gelatin, the reaction associated with this being pH 4·8—5·1. As the concentration of gelatin increases above 2 per cent., the turbidity decreases, 8 per cent. gels being just opalescent and 10 per cent. gels quite transparent. Similarly, when the concentration is reduced to 0·2—0·1 per cent. gelatin, the sols are but slightly opalescent. In the concentration region 2·0—0·2 per cent. gelatin, the systems are unstable in the pH zone, 4·8—5·1, and flocculation of the gelatin results, most rapidly in systems of 1 per cent. gelatin.

Miss Pleass interprets the phenomena, as does Ostwald,[2] "by postulating the change on cooling from a solution of gelatin in water to a solution of water in gelatin with the formation of a framework of hydrated gelatin" (see p. 104).

THE PROBLEM OF GEL STRUCTURE

According to Hatschek [3] the term "gel" describes "transparent or translucent bodies containing a considerable fraction of liquid, but maintaining their shape and exhibiting rigidity."

Numerous investigators have approached the problem of the mechanism of the sol ⇌ gel transformation and the structure of gels. Several excellent summaries of the work in this field have recently been given, and for details of early theories the student should refer to these.[4]

SOL ⇌ GEL TRANSFORMATION

The question of the retention of structure in some measure when a gelatin gel is melted to the sol form has been the subject of many inquiries. As early as 1909, Menz [5] showed that under the ultramicroscope 0·5 per cent. gelatin sols were optically empty, but on

[1] *Proc. Roy. Soc.*, **A 126,** 416 (1930).
[2] *Loc. cit.*, p. 33.
[3] *Chem. and Ind.*, **48,** 389 (1929).
[4] Miss Lloyd, Alexander's "Colloid Chemistry" (New York, 1926), Vol. 1, 767—82; Bradford, *ibid.*, 751—66; Ostwald, *Kolloid-Z.*, **46,** 248 (1928); Bogue, *J. Am. Chem. Soc.*, **44,** 1343 (1922); Hatschek, *J. Soc. Chem. Ind.*, **48,** 389 (1929).
[5] *Z. physik. Chem.*, **66,** 129 (1909).

cooling there was a gradual appearance of submicrons, whose size gradually increased. Similarly, Arisz [1] noticed an increasing intensity of the Tyndall phenomenon on cooling.

The possibility of a transition temperature for sol $\rightleftharpoons$ gel reversal immediately presents itself, and Smith [2] investigated this by studying the mutarotation of gelatin solutions, concluding that gelatin probably exists in two forms : sol form A, stable above 33°—35° C., and gel form B, stable below 15° C. He proposed a bimolecular reaction between the temperatures just named ; both forms coexist between 15° C. and 35° C. The fact that the optical rotation increases as the viscosity increases is believed to indicate increasing formation of the gel form. Davis and Oakes [3] later found 38·03° C. to be the transition temperature.

Investigating the viscosities of dilute (less than 1 per cent.) solutions of ash-free gelatin at 20° C. and 25° C. Fuseya, Sasaki and Nazano [4] observed that there is a step in the concentration-viscosity curve at a concentration of about 0·1 per cent., depending on the variety of gelatin. Again, measurement of the electrical conductivity of the solutions revealed a minimum at a concentration a little less than corresponded to the step in the concentration-viscosity curve. These and other results are explained on the basis of Smith's sol $\rightleftharpoons$ gel equilibrium. The authors emphasise that even in very dilute solutions, gel structure exists in some degree and of the same nature as in more concentrated systems. As the concentration increases, a point is reached where there is the minimum amount of gelatin necessary for gelation. Hence, at temperatures below 35° C., where the gelatin is in the gel form, the viscosity will not change with time if there is not sufficient of the gel form present. The step in the concentration-viscosity curve corresponds to the minimum amount of gelatin necessary to permit gelation of the system.[5] Above 35° C. all the gelation is in the sol form and gelation cannot take place. The step curve only results when the pH value of the gelatin solution is less than the isoelectric point, as otherwise the gelatinising power of gelatin is reduced.

It has been suggested [6] that gelatin exists in two forms : (*a*) sol, stable at 40° C. ; (*b*) gel, stable at 0·5° C. The sol form is considered to consist of molecules with probably a chain type of linkage between the amino acids, whereas the gel form is assumed to consist

[1] *Kolloidchem. Beihefte*, **7**, 1 (1915).

[2] *J. Am. Chem. Soc.*, **41**, 135 (1919). Tsuda, *Kolloid-Z.*, **47**, 28 (1929), confirms the critical temperature range 33°–35° C., but suggests that instead of chemically different entities, the phenomenon is one of difference in degree of dispersion, the gelatin forming hydrated micelles.

[3] *J. Am. Chem. Soc.*, **44**, 464 (1922).

[4] *J. Phys. Chem.*, **34**, 2012 (1930).

[5] Cf. Rohloff and Shinjo, *Physik. Z.*, **8**, 442 (1907) who observed that the elasticity of a gelatin solution becomes evident at 0·18 per cent. concentration, which is correlated with the formation of gel structure.

[6] Carpenter, Dahlberg and Hening, *Ind. Eng. Chem.*, **20**, 397 (1928).

of a molecule containing some linkages of the ketopiperazine type.[1]

Rettig [2] found that heating gelatin solution caused the irreversible production of a non-gelatinising gelatin, termed by Traube,[3] β-gelatin. This is accompanied by an increase in the electrical conductivity. The velocity of the transformation increases with the duration of heating and with increase in temperature; it is greater in dilute solutions.

Frankel [4] has investigated the effect of prolonged heating on the disintegration of gelatin in solution into smaller and non-colloidal units. Heating at 36·5° C. results in a lower viscosity, loss of N on dialysis, gradual loss of capacity to gel when cooled, and a decrease in optical rotation. He later obtained further evidence [5] for the disintegration to smaller units by the fact that such gelatin is more rapidly hydrolysed by pepsin. Not only incubation at 36°—37° C., but also exposure to sunlight was found by Frankel [6] to produce a breakdown to smaller units in gelatin solutions. A marked reduction in viscosity was noted, and on dialysis these solutions rapidly lost N. After warming a 2 per cent. solution for 144 hours, the sol refused to set again at 10° C.[7]

The recent work of Neville, Theis and Oswald [8] shows that gelation temperature and melting point are not constants but vary with the thermal history. Using a modified dilatometer whereby gelation and melting temperatures of gelatin sols could be determined under very carefully controlled conditions, continuous readings were possible without disturbance of the gel structure. The curve obtained when warming a 10 per cent. gel is not the same as that given by the cooling curve and the melting temperature is always higher than the gelation temperature, *i.e.*, hysteresis is exhibited.

All cooling curves of the gelatin sols showed a sudden change in slope at about 36° C., and the authors attribute this to the transformation of sol form A into gel form B at this temperature. "The inflection indicates a decrease in the hydration of the form stable at the lower temperatures, and is probably due to a decrease in the degree of dispersion of the gelatin at the lower temperatures."

There was found no evidence of any connection between jelly strength and the gelation, melting or transition temperatures, although, in general, a higher melting point accompanies higher jelly strength.

[1] In this connection see Levene and Pfaltz, *J. Biol. Chem.*, **63,** 661; **68,** 277 (1925); *J. Gen. Physiol.*, **8,** 183 (1925).
[2] *Kolloid-Z.*, **27,** 165 (1920).
[3] *Reichart's and du Bois Reymond's Arch.* (1867), p. 87; cf. Gerike, *Kolloid Z.*, **17,** 84 (1915).
[4] *Z. physiol. Chem.*, **167,** 17 (1927).
[5] *Ibid.*, **170,** 247 (1927).
[6] *Kolloid-Z.*, **45,** 355 (1928).
[7] Cf. von Schroeder, *Z. physik. Chem.*, **45,** 109 (1903).
[8] *Ind. Eng. Chem.*, **22,** 60 (1930).

The mechanism of the sol $\rightleftharpoons$ gel transformation has been explained by Fanselow [1] on the basis of changes in the electrokinetic potential of the gelatin micellæ. Low electrokinetic potential on these micellæ allow their attraction for one another to be more pronounced, and such attraction together with their attraction for the dispersion medium, means a high resistance to shear, and an accompanying increase in optical activity.

Marinesco [2] measured the specific inductive capacity of gelatin solutions and found it to rise rapidly in linear relation to the concentration, a sharp maximum appearing at 0·6 per cent. concentration, followed by a rapid decrease. This maximum is coincident with the minimum concentration favourable to gelation. Below this critical concentration the solutions show no Tyndall cone, no viscometric rigidity and act as excellent protective colloids for hydrophobe sols. Marinesco believes that the gelatin molecule is more highly polar than water molecules, and that in concentrations less than 0·6 per cent. the gelatin is present as single molecules. Aggregation to units having zero electric moment occurs at higher concentrations.

In a study of the ageing of gelatin solutions, Nagorny [3] measured the viscosity of gelatin solutions maintained from 4 to 8 weeks at (*a*) 37° C. and (*b*) 6° C. The viscosity of (*a*) decreased and of (*b*) increased with age, all viscosity measurements being determined at 20° C. When the viscosity was determined at 30° C., both (*a*) and (*b*) gave approximately the same results. At 30° C. both (*a*) and (*b*) approximately obey Poisseuille's law, but at 20° C. (*b*) is very widely out, although (*a*) obeys approximately.

The precipitation of gelatin by means of $(NH_4)_2SO_4$ was next investigated, and it was found that different amounts of this salt were required for (*a*) and (*b*). By ageing warm, more sulphate is necessary than for a much longer ageing in the cold. If a warm-aged sol is now held at 6° C., the quantity of sulphate required for precipitation is again reduced. Through fractionation with $(NH_4)_2SO_4$ a cold-aged sol was divided into two fractions of different viscosity, only one of which gelatinised at room temperature. Two possibilities arise, assuming that in both cases cooling produces coarser disperse particles, which are responsible for jellying, and that although at 20° C. these persist, they become more finely dispersed at 30° C. The first possibility is that the coarse and fine particles are chemically identical and differ only in degree of dispersity. The second possibility is that they are chemically different and Nagorny accepts this on the basis of the $(NH_4)_2SO_4$ experiments. Hatschek,[4] after reviewing in some detail the known facts concerning gelatinisa-

[1] *Am. Coll. Symp. Monog.*, **6,** 250 (1929).
[2] *Compt. rend.*, **187,** 718 (1928); **188,** 1163 (1929).
[3] *Kolloid-Z.*, **41,** 123 (1927).
[4] *Chem. and Ind.*, **48,** 398 (1929).

tion, reaches the conclusion that the particles in the sol and the gel of gelatin are not identical (see p. 88).

THE NATURE OF GELATIN SOLUTIONS

Sheppard and his co-workers [1] obtained data on viscosity, which point to the fact that gelatin in aqueous solutions is molecularly dispersed, " at any rate in the sense that every part of the molecule is exposed to the solvent." Probably the micellæ in gelatin solutions at the necessary temperature and dilution form " macromolecules of elongated form but with some degree of branching of the chains " with a tendency to clustering and association, increasing as the temperature falls and dilution increases. The macromolecules have molecular weights probably ranging from 10,000 to 30,000 (see p. 64).

Most gelatins probably contain a proportion of incompletely peptised collagen particles and hydrolysis of this collagen leads to an initial increase in viscosity and gelatinising power, followed by a fall as the gelatin hydrolysis progresses, the mean molecular weight also falling steadily.

X-ray investigations [2] support Sheppard's views that " the protein collagen consists of fibres built up of crystallites composed of primary valence chains of the anhydro-amino-acids, the protein macromolecules." The peptising of these fibres yields gelatin, the crystallites and primary valence chains being disoriented and separated. Combination of water (hydration) and chain complexes occurs but the mutual attraction of these macromolecules, as well as their capacity to bind water, become reduced as the chains are shortened by hydrolysis, and the final result is simply a mixture of amino-acids.

Investigations in Sheppard's laboratory point to the solution unit in dilute gelatin solutions as having one dimension of " low molecular " thickness, viz., 7ÅU., in good agreement with the findings of Gorter and Grendel.[3] Although in dilute solutions such entities may form the final structure units, in more concentrated solutions aggregation results in multimolecular micelles " with very probable approach to nematic and smectic ordering in the micelles and ' swarms.' "

GELATION

Assuming that gelatin is formed from collagen by a process of disorientation of the macromolecules of collagen crystallites,

[1] Sheppard, *Nature*, **107**, 73 (1921); Sheppard and Sweet, *J. Am. Chem. Soc.*, **44**, 2797 (1922); Sheppard and McNally, *Am. Coll. Symp. Monog.*, **7**, 17 (1930); Sheppard and Houck, *J. Phys. Chem.*, **34**, 294 (1930).

[2] Meyer and Mark, *Ber.*, **61**, 693 (1928).

[3] *Trans. Faraday Soc.*, **22**, 477 (1926).

Sheppard and McNally [1] believe gelation to be an incomplete recrystallisation in which the aggregation or condensation process is more advanced than the orientation process. This conception may be compared with that of Reiger [2] who has made a detailed examination of the view that gelation is not a single process but is the consequence of a series of processes developed independently of each other. Three methods of investigation were used : (1) change in viscosity with time, (2) change in elasticity with time, and (3) change in optical rotation with time.

Since rotation and viscosity show no variation above about 45° C., but do so on cooling, Reiger concludes that molecular transformations take place, gelation being a series of reversible monomolecular reactions, yielding continually more complex molecules.

Assume that at 45° C. only molecules of genus G_0 exist ; at lower temperatures they are transformed to genus G_1, which in turn are transformed into higher complexes, accompanied by a gradual transition from the liquid to the solid state. A detailed mathematical exposition is built on the series of single reactions :

$$v_1G_0 \rightleftharpoons G_1,\ v_2G_1 \rightleftharpoons G_2 \ldots\ldots v_1G_{i-1} \rightleftharpoons G_i \ldots\ldots v_nG_{n-1} \rightleftharpoons G_n$$

Gelation involves physical changes as well as chemical changes.

Regarding the rate of gelation, Shoji [3] has shown that the longer the duration of heating a gelatin solution, the lower is the initial velocity of gelation. He found that the change in viscosity of a gelatin sol with respect to time when gelation is incipient is :

$$\eta - \eta_1 = \mu^t + t\left(\frac{\mu}{\lambda}\right),$$

where η = viscosity at time t.

η_1 = viscosity at time of initial gelation (by extrapolation).

λ and μ = constants.

Such an empirical equation serves to represent gelation for gelatin sols, but not for agar-agar, where viscosity increases at a diminishing rate with time finally attaining a constant value, which in the case of very dilute agar-agar sols is proportional to the concentration but which rises greatly as the concentration increases.[4]

That the setting of gelatin sols to gels is greatly influenced by the presence of acids or alkalis is generally recognised and recent very careful work in this connection has been carried out by Miss Pleass.[5] Coignet's " Gold Label " leaf gelatin was purified [6] so that the ash content varied from 0·02 to 0·01 per cent. Fig. 12 reproduces

[1] *Loc. cit.*
[2] *Kolloidchem. Beihefte*, **19,** 381 (1924).
[3] *Biochem. J.*, **13,** 227 (1919).
[4] Hirota, *J. Biochem. Tokyo*, **9,** 103 (1928).
[5] *Proc. Roy. Soc.*, **A. 126,** 406 (1930).
[6] Jordan-Lloyd and Pleass, *Biochem. J.*, **21,** 1352 (1927).

Miss Pleass' setting curves, indicating the minimum concentration of gelatin, which will form a gel at temperatures of 0°, 12°, 18° and

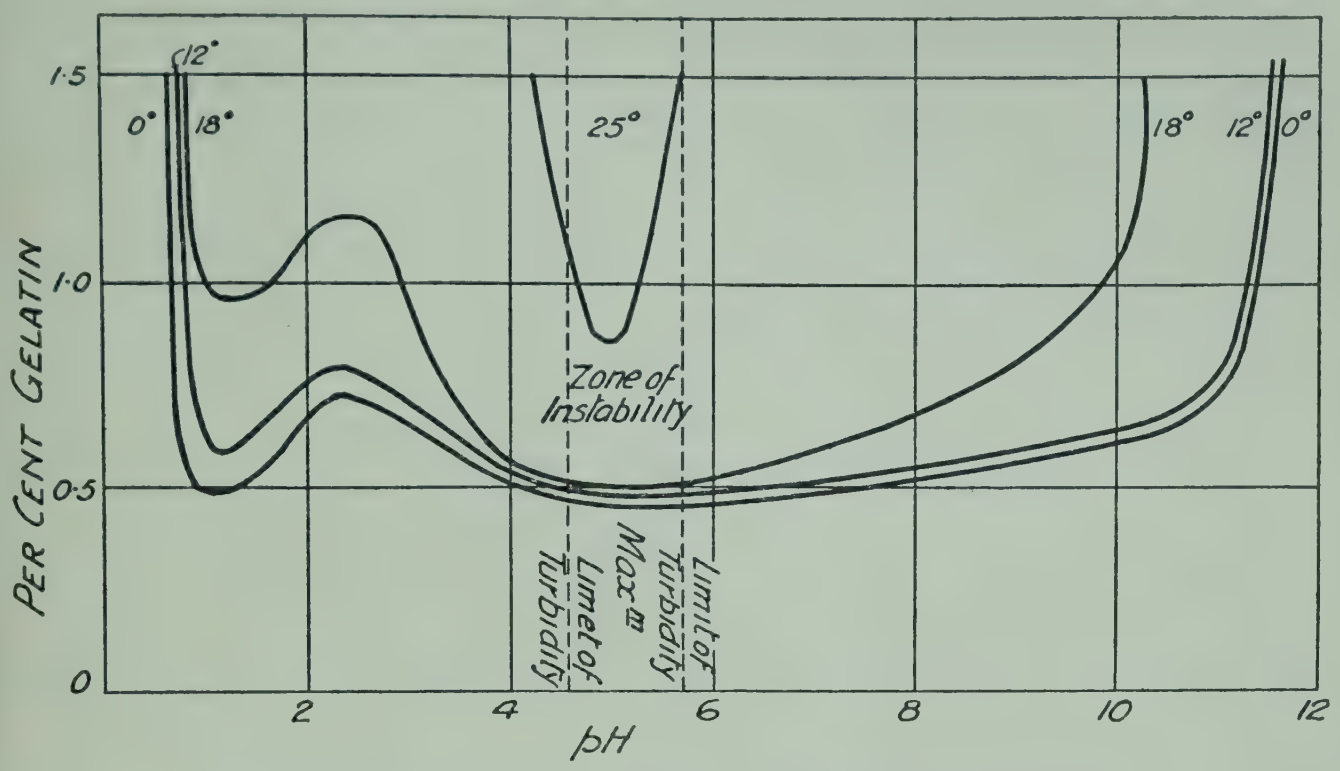


FIG. 12.—The setting of gelatin sols in relation to pH.

25° C. over a pH range 0·5 to 11·5. From these curves the following data and remarks were drawn :

SETTING CURVES FOR GELATIN SOLUTIONS

	25° C.	18° C.	12° C.	0° C.
Acid Zone, pH 0·5–4·0.	Steep gradient between pH 4·8–4·2. No gel formation at acidity greater than pH 4·2.	Broad maximum in zone pH 2·3-3·6 (gelatin = 1·2 per cent.). Setting is inhibited at pH< 0·9.	Similar to 18°C., but less gelatin is required, *e.g.* 0·8 per cent. at pH 2·3.	Similar to 12° C. At pH 2·3 the minimum gelatin is 0·75 per cent.
Alkaline Zone, pH 6·0–11·5.	Very steep gradient. No gels at pH>5·6, probably owing to hydrolysis.	Steep gradient Gelatin is inhibited at pH >10·2.	The minimum gelatin concentration for setting increases very slightly with increasing alkalinity from pH 5–10. From pH 10–11 the curve is steep and gelation demands increased gelatin concentration. No gel at pH>11·5.	
Isoelectric Zone, pH 4–6.	Sols unstable between pH 4·5–5·5. Turbid white flocculent precipitation from sols of 0·2–0·5 per cent. gelatin. Gels only possible with 0·9 per cent. or more gelatin and very sensitive to pH change, *e.g.*, 1·5 per cent. sols only set in pH zone 4·2–5·5.	More concentrated solutions form gels, their rigidity increasing with concentration ; usually there is flocculation within 48 hours from sols containing less than 2 per cent. gelatin.		

THE STRUCTURE OF THE GEL

Evidence for the existence of a structure in gelatin gels is afforded by the work of Callow [1] on the crystallisation of ice in supercooled gels, and of Ruhland [2] who found that while crystalloidal dyes (*e.g.*, methylene blue) diffused through gels very readily, colloid dyes (*e.g.*, Bismarck brown) could not penetrate the gel.[3]

The problem of the structure of the gel has exercised the minds of numerous investigators, and even now there is no one generally accepted theory. The fact that the sol $\rightleftharpoons$ gel transformation is thermally reversible must be kept in mind, together with the fact that the electrical conductivity of a gel differs but little from that of the corresponding sol. Again, Abramson [4] has lately shown that particles of quartz, zinc and mercury have the same cataphoretic mobility in *soft* 1 per cent. gelatin gels as in the sols, this being a function of the true viscosity of the sol or gel and proportional to the applied E.M.F., and unchanged by the presence of gel structure. The modern theories of gel structure fit in with such observations which point to the existence of only slight differences in the physical condition of sols and their corresponding gels.[5]

The older theories of gel structure (Frankenheim, Nägeli, Bütschli) considered gels to consist of a loosely-bound network of molecular aggregates or submicrons. Van Bemmelen's observations[6] on silicic acid hydrogels lent great support to this view of cellular structure.[7] Quincke [8] advanced an emulsion theory [9] of gels, *i.e.*, they contain two phases, the liquid being rich and poor in colloid material respectively. Hatschek [10] has shown the emulsion theory to be untenable, but Ostwald [11] has recently supported the view of a two-liquid structure as possible with certain gels and distinguishes between lyogels (rich in liquid) and xerogels (relatively dry).

Passing over the views held by Zsigmondy,[12] Sutherland,[13] Garrett [14] and Bachman,[15] we find that investigators incline to the

[1] *Proc. Roy. Soc.*, **B. 108,** 307 (1923).
[2] *Jahrb. wiss. Botan.*, **51,** 376 (1912).
[3] The Liesegang ring phenomenon may also be invoked in this connection; cf. Broderson, *Kolloid-Z.*, **35,** 21 (1924).
[4] *J. Gen. Physiol.*, **11,** 743 (1928).
[5] *Vide* Wo. Ostwald, *Arch. ges. Physiol.*, **109**, 277 (1905); **111,** 581 (1906); Walpole, *Kolloid-Z.*, **13,** 241 (1913); Stiles and Adair, *Biochem. J.*, **15,** 621 (1921); Stiles, *Proc. Roy. Soc.*, **A. 103,** 261 (1923).
[6] *Z. anorg. allgem. Chem.*, **13,** 233 (1896); **18,** 14 (1898).
[7] *Vide* Robertson, " Physical Chemistry of the Proteins " (1918), p. 325.
[8] *Sitzb. kgl. preuss. Akad. Wiss.* (1901), 858.
[9] Cf. Hardy, *Z. physik. Chem.*, **33,** 326 (1900); *Proc. Roy. Soc.*, **66,** 95 (1900); Jordan-Lloyd, *Biochem. J.*, **14,** 147, 584 (1920).
[10] *Trans. Faraday Soc.*, **12,** 17 (1916).
[11] *Kolloid-Z.*, **46,** 248 (1928).
[12] *Physik. Z.*, **14,** 1098 (1913); " Physics and Chemistry of Colloids " (London, H.M. Stationery Office, 1921), p. 51.
[13] *Proc. Roy. Soc.*, **B. 79,** 130 (1907).
[14] *Phil. Mag.* (6), **6,** 374 (1903).
[15] *Kolloid-Z.*, **23,** 85 (1918); **11,** 145 (1912).

view that gelation bears some resemblance to crystallisation. Thus, Procter,[1] arguing from the ultramicroscopic revelation that a gelatin sol on cooling coalesces into flocculent masses, stated that gels are probably networks of minute and, possibly, fibrillar, flexible crystals. Thompson [2] held similar views, as did Moeller,[3] who suggested that gelatin is an irregular aggregation of fibrils, which form a network of α-gelatin with β-gelatin occupying the spaces therein. Removal of β-gelatin by any means renders the structure visible, as orientation of the α-gelatin fibrils occurs and the fibril aggregates become visible under the ultramicroscope. Bradford [4] argues that gels are two-phase, liquid-solid systems in which are numerous submicroscopic crystalline aggregates, the mother liquid from which the solid disperse phase has crystallised being held in and around the aggregates by molecular and capillary forces. The separation of gelatin from solution and its crystallisation are treated from the standpoint of von Weimarn's formula.[5] McBain [6] using the data from his extensive work on soap systems, believes that " all the characteristic properties of gels can be explained in the light of the micellar theory," *i.e.*, the theory that the structure is fibrous and made up of particles or micelles. Such particles stick together, very probably in thread-like strings.

Kraemer [7] agrees that " gel formation is the result of an incomplete or unsuccessful attempt at precipitation of a solid phase from a liquid system, and Miss Lloyd [8] is of similar opinion, the one fundamental rule for all gels being that they are derived from two components, one being liquid at the temperature in question and the other (the " gelator ") being solid. The rigidity and elasticity of gels are considered to be due to the presence therein of a highly-dispersed phase, solid or semi-solid in character, so united together as to form a structure of the solid as framework. Accordingly, only dispersed systems can yield gels, and the gel state is immediately preceded by the sol condition.

Working in Miss Lloyd's laboratory, Miss Pleass [9] has just summarised the physical conditions obtaining in gelatin sols and gels, with special reference to concentration :—

(A) **Gelatin Concentration** $>$ 10 **per cent.**—At fairly high temperatures the sols are solutions of small, lightly-hydrated gelatin aggregates in water. On cooling, there is absorption of most of the

[1] *J. Am. Leather Chem. Assocn.*, **12,** 536 (1917).
[2] *J. Soc. Leather Trades' Chem.*, **3,** 209 (1919).
[3] *Kolloid-Z.*, **19,** 213 (1916).
[4] *Science Progress*, **12,** 63 (1917); *Biochem. J.*, **12,** 351 (1918); **14,** 91 (1900); **15,** 353 (1921); Alexander's " Colloid Chemistry " (New York, 1926), Vol. 1, p. 751.
[5] " Zur Lehre von den Zustanden der Materie " (Dresden, 1914).
[6] Adhesives Res. Comm., *Rept.* **1,** 129 (1922).
[7] *J. Phys. Chem.*, **29,** 1523 (1925); *Am. Coll. Symp. Monog.*, **4,** 110 (1926).
[8] Alexander's " Colloid Chemistry," Vol. 1, 767.
[9] *Proc. Roy. Soc.*, **A. 126,** 425 (1930).

water available for the formation of gelatin hydrates, thus filling the media to an extent that their coherence leads to a solid framework of hydrated gelatin. Hence, the sol is a solution of gelatin in water, whilst the gel is a pseudo-solution of water in gelatin.

(B) **Gelatin Concentration 2–10 per cent.**—The sols show similar behaviour, but their greater dilution leaves more water available than is necessary for hydrate formation. Cooling leads to a gelatin framework, which "probably consists of gelatin-water aggregates of large dimension, permeated by water, and as long as water in excess of that proportion which may be held mechanically by capillary attraction within the gelatin framework is not present, the framework of the gel is stable."

(C) **Gelatin Concentration 0·5–2 per cent.**—On cooling there arises a metastable gel framework, the interstices filled with the excess water. Due to the osmotic forces being at a minimum, the water cannot support the gelatin framework and the gelatin sinks out as a clot. Weaker sols on cooling fail to form gels, as there are insufficient gelatin aggregates to cohere and form a framework throughout the medium.

Miss Pleass points out that when acid or alkali is added to the system, there is a disturbance of the balance of forces between the water molecules and the gelatin aggregates, and less water combines with the gelatin than is the case in isoelectric systems. However, due to osmotic forces, a large volume of water is held in the interstices of the gel framework, the osmotic force acting in opposition to the rigidity of the gel.

The question of the microstructure of gelatin gels and the order of magnitude of the discontinuities in them has been raised by Kraemer.[1] That the structure is rather fine-grained follows from the optical behaviour and general properties of gels. However, the vapour pressure of water over a weak gel, together with the somewhat easy diffusion of ions and molecules through a gel, show that the high rigidity of the system in bulk is not retained on a molecular scale. Svedberg [2] had suggested that a study of the Brownian motion of very small particles embedded in weak gels might throw light on the structure of gels. Kraemer followed this up by preparing particles of mercury by electrical pulverisation [3] and roughly fractionated by sedimentation. He employed particles of 200—250 milli-microns radius and mixed them with dilute (0·3 per cent.) gelatin solutions before gelation. Cinematograph records of the Brownian motion showed that "the structure of such weak gelatin gels is still considerably finer than the indicating mercury particles and their displacements; the system behaved as a very viscous

[1] *J. Phys. Chem.*, **29,** 1523 (1925); cf. Freundlich, *Ber.*, **61 B.** 2219 (1928).
[2] *Am. Coll. Symp. Monog.*, **1,** 93 (1923).
[3] Cf. *J. Am. Chem. Soc.*, **46,** 1890 (1924).

'homogeneous' liquid." At the same time, a quite considerable resistance to Brownian motion was observable.

An extension of the investigation [1] aimed to explore the region of discontinuities between the scale of Brownian motion and molecular size by a study of the diffusion of molecules into and through the gels. Using urea, glycerin and sucrose, it was found that increasing the concentration of the gel caused diffusion rapidly to slow down. By setting up a relation connecting the diffusion constants in water and in the gel so as to include the radii of the openings in the gel frame-work the authors obtained the following values for the pore size of different gels :—

Diffusate.	Radius of Pores in Millimicrons.		
	5 Per cent. Gel.	10 Per cent Gel.	15 Per cent. Gel.
Sucrose . . .	5·5	1·4	0·5
Glycerin . . .	5·7	1·7	1·0
Urea	4·7	1·5	0·8

Friedman [2] next examined the specificity of the diffusing non-electrolyte. Studying the effect of non-electrolytes upon the diffusion of urea into a 4·7 per cent. gelatin gel from a 3 per cent. solution, there was observed a very specific action on the diffusion rate by various non-electrolytes, *e.g.*, sucrose and glucose greatly increased the rate, while methanol effected a pronounced decrease. Thus these non-electrolytes exert some effect on the gel structure.

The temperature of setting and the age of the gel influenced the rate of diffusion, gels set at lower temperatures offering less resistance to diffusion than gels set at higher temperatures. Friedman interprets this as indicating a more open structure, an argument agreeing with Hardy's observation that a gel structure is the finer the more rapidly the gel is cooled.[3]

Barratt,[4] who has done considerable work on gels, inclines to the view that a gel consists of intersecting fibrils running perfectly straight and united at their points of intersection. His experimental work showed that these fibrils are already in existence for a relatively considerable time before they become visible and they are initially amicroscopic, later increasing in size to ultramicroscopic visibility. A diameter of 1μ is sometimes reached. A "dry" rigid gel is understood to contain numerous and very fine fibrils, giving a gel with an enormous resistance to the passage of water

[1] Friedman and Kraemer, *ibid.*, **52,** 1295 (1930).
[2] *Ibid.*, **52,** 1305 (1930).
[3] *J. Physiol.*, **24,** 288 (1899).
[4] *Biochem. J.*, **14,** 189 (1920); "Physics and Chemistry of Colloids" (London, 1921), p. 49.

through the interstices ; when fibrils are coarse and relatively few, there results a moist gel with syneresis and a certain amount of elasticity.

Similarly, Briefer and Cohen [1] postulate an elastic fibrillar structure for gels. Imbibition swells the individual fibres and coherence results. On heating a gel, the fibrils shrink and the imbibed water is excreted. Gradually the threads curl up and separate, and, with rising temperature, they contract into spirals, ever more tightly so that finally they approximate to spherites. Under suitable time-temperature conditions the elasticity of the fibrils is not impaired and a reversible sol $\rightleftharpoons$ gel transition is possible. Excess time and/or temperature causes the spherite condition to be retained and as the elastic limit of the threads is exceeded, a flocculent suspension results, and cooling now fails to re-establish the original fibril form. From these premises the authors believe that jelly strength is a function of the relative volume and number of individual fibrils present in the gel condition, while viscosity is a function of their thickness and length in the sol condition.

Excellent experimental work has been carried out by Poole,[2] who supports the idea that gels are two-phase, solid-liquid systems. He deduced a mathematical formula to satisfy the requirements of a gel structure, where the solid phase would possess the form of a mesh of cylindrical fibrils or threads. Poole's theory is that the fibrils are formed from a material which is in dynamic equilibrium with the water of the liquid phase " as a result of either a reversible hydrolysis or hydration whereby the ratio of the gelatin in the solid phase to that in the liquid phase becomes progressively less with rising temperature." Since the chief difference between sols and gels resides in the elasticity of the latter, Poole investigated the elasticity-temperature-concentration relations of gelatin gels, and his findings support his mathematical deductions from the assumed fibrillar structure.

SYNERESIS

Syneresis is the phenomenon of the separation of fluid from a gel and is particularly evident with agar-agar gels, as bacteriologists are aware.[3] The phenomenon is met with in such diverse instances as the breaking of rennin-clotted milk (junket), the ageing of starch pastes and the serum exudation from blisters and wounds.

Kuhn [4] has critically examined important published data relating

[1] *Ind. Eng. Chem.*, **19,** 252 (1927).

[2] *Trans. Faraday Soc.*, **21,** 114 (1925). *Vide* also, Hatschek, *Chem. and Ind.*, **48,** 396 (1929) ; *Kolloid-Z.*, **36,** 49 (1925) ; Scarth, *J. Phys. Chem.*, **29,** 1009 (1925) ; Sauer and Kinkel, *Z. angew. Chem.*, **38,** 413 (1925).

[3] Cf. Healy, *J. Bact.*, **12,** 179 (1926).

[4] *Kolloid-Z.*, **46,** 299 (1928).

to syneresis and believes the phenomenon can be approached from two sides of a hydration equilibrium : gelation and swelling. Thus :—

Zerogel ⇌ sol formation ⇌ gelation ⇌ syneresis ⇌ swelling ⇌ Zerogel
+ liquid + liquid.

Whilst certain gels (silicic acid, caoutchouc) exhibit more intense syneresis as the concentration is increased, others (starch, agar-agar) do so in more dilute systems. Thus, Meyer [1] showed that the syneresis of arrowroot starch pastes at 37° C. began after 10 days and 11 days for 6 per cent. and 7 per cent. concentrations respectively, but weaker pastes showed much earlier syneresis.

The influence of temperature on syneresis cannot be formulated in a general rule, since hydration and temperature do not run parallel and because the thermal history of the preparation plays a significant *rôle*. Syneresis varies in amount with the nature of the dispersion medium and with the application of pressure. Kuhn shows that syneresis reaches a maximum when the gel in question is least stable, *e.g.*, with gelatin at its isoelectric point. His discussion concludes with the opinion that syneresis marks the breakdown of the gel form by alterations in structure and by desolvation.

In a series of papers, Liepatoff [2] advances the theory that syneresis is the continued operation of the forces causing gelation, *i.e.*, the operation of mutually attractive forces between the particles of the disperse phase in close proximity. Greater attraction between the particles squeezes out the liquid between them, the velocity of such syneresis increasing with concentration. This is similar to Hardy's [3] view that syneresis occurs when the attraction of the dispersed phase for itself is greater than its attraction for the medium.[4]

SYNERESIS OF GELATIN

Miss Lloyd [5] connects the syneresis of gelatin gels with the disturbance of the equilibrium between colloidal gelatin ions and diffusible ions of the same charge, which results when the swollen gel is removed from its surrounding solution to an atmosphere saturated with water vapour. Expulsion of diffusible ions from the gel, together with the corresponding ions of opposite charge, weakens the osmotic forces in the gel and water is squeezed out due to the elastic recoil of the gel. Syneresis " is probably due in all cases to

[1] *Kolloidchem., Beihefte*, **5**, 1 (1913).
[2] *Kolloid-Z.*, **41**, 200 (1927) ; **43**, 396 (1927) ; **48**, 62 (1929) ; **49**, 321 ; 441 (1929) ; *Biochem. Z.*, **192**, 91 (1928).
[3] *Kolloid-Z.*, **46**, 268 (1928). Cf. Lewis and Daniel, *Am. Coll. Symp. Monog.*, **4**, 130, (1926).
[4] But see Prakash and Dhar, *J. Indian Chem. Soc.*, **7**, 417 (1930).
[5] " Chemistry of the Proteins " (London, 1926), p. 180.

the osmotic forces leading to extension being greater than the elastic forces leading to recoil." Miss Lloyd's work in this field showed that a very pure gelatin does not yield a stable gel in the isoelectric zone, inasmuch as over this pH range " the elastic framework of the gel is not counter-balanced by osmotic pressure," and its unhindered contraction causes the separation of fluid from 1 per cent. gels, the gelatin simply retaining its water of imbibition.

An extension of these observations has been made by Kunitz. A 4 per cent. solution of isoelectric gelatin, in complete absence of free water, shows very slow syneresis, whereas a trace of water, directly added or gained by squeezing, sets up a rapid diffusion of water from the gel. Kunitz [1] develops the idea that the force operating in the contraction of a dilute gel is connected with the micellar structure of the gelatin solution, and has its origin in the strain resident in the micellæ prior to the gelatin setting to a gel. This strain arises from the osmotic pressure of the soluble portion within the micellæ and is less as the total concentration of gelatin increases.

Cooling a gelatin solution leads to the adherence of micellæ, forming fibrils, which interlace to form a network occluding gelatin solution. If electrolytes are absent gelatin is precipitated and the micellæ shrink and lose water. Such shrinkage slows up in the solid (gel) state, since the diffusion of the water is retarded mechanically. On contact with water, however, diffusion is at once enhanced. There is shrinkage of each individual micelle, the whole network contracting, and not only is the water that was inside the micelle expelled, but a considerable amount that was held between the micellæ by capillary forces.[2]

THE OPTICAL ROTATION OF GELATIN

The investigation of the optical rotation of gelatin in solution is important as an index to the colloidal behaviour of the system. Trunkel [3] observed that the rotation decreased as the temperature was raised, reaching a minimum value at 30°—35° C., after which no change occurred. Fig. 13 is reproduced from results by Reiger [4] and shows the convergence of the temperature—specific rotation curves corresponding to time intervals of 3, 6, 10, 15, 30, 60 and 120 minutes. Convergence occurs at about 35° C. Most of the earlier work can be passed over in favour of the extensive research of Kraemer.[5]

Kraemer employed 0·47 per cent. solutions of de-ashed gelatin

[1] *J. Gen. Physiol.*, **12,** 289 (1928).
[2] Northrop and Kunitz, *J. Phys. Chem.*, **35,** 172 (1931).
[3] *Biochem. Z.*, **26,** 493 (1910). Discussed by Wo. Ostwald, *Kolloid-Z.*, **17,** 113 (1915).
[4] *Kolloidchem. Beihefte,* **19,** 400 (1924).
[5] *Am. Coll. Symp. Monog.*, **4,** 112 (1926).

and studied the relation between the specific rotation and temperature and pH value. He obtained two forms of curves : (1) at 27·5° C. and above, the specific rotation has minimal values at pH 5 and pH 8—9, although the general variation with pH value is not pronounced ; (2) at 25° C. and below, the minima of the higher temperatures are replaced by maxima, except in a very narrow region about pH 4·95 characterised by a sudden rise in the Tyndall effect. If a thin enough layer be used (to prevent the masking of observations by the intense turbidity) there is observed an actual minimum in the specific rotation.

As the temperature falls, the specific rotation and gel-forming capacity increase in the zone pH 5—8, partially masking the minor

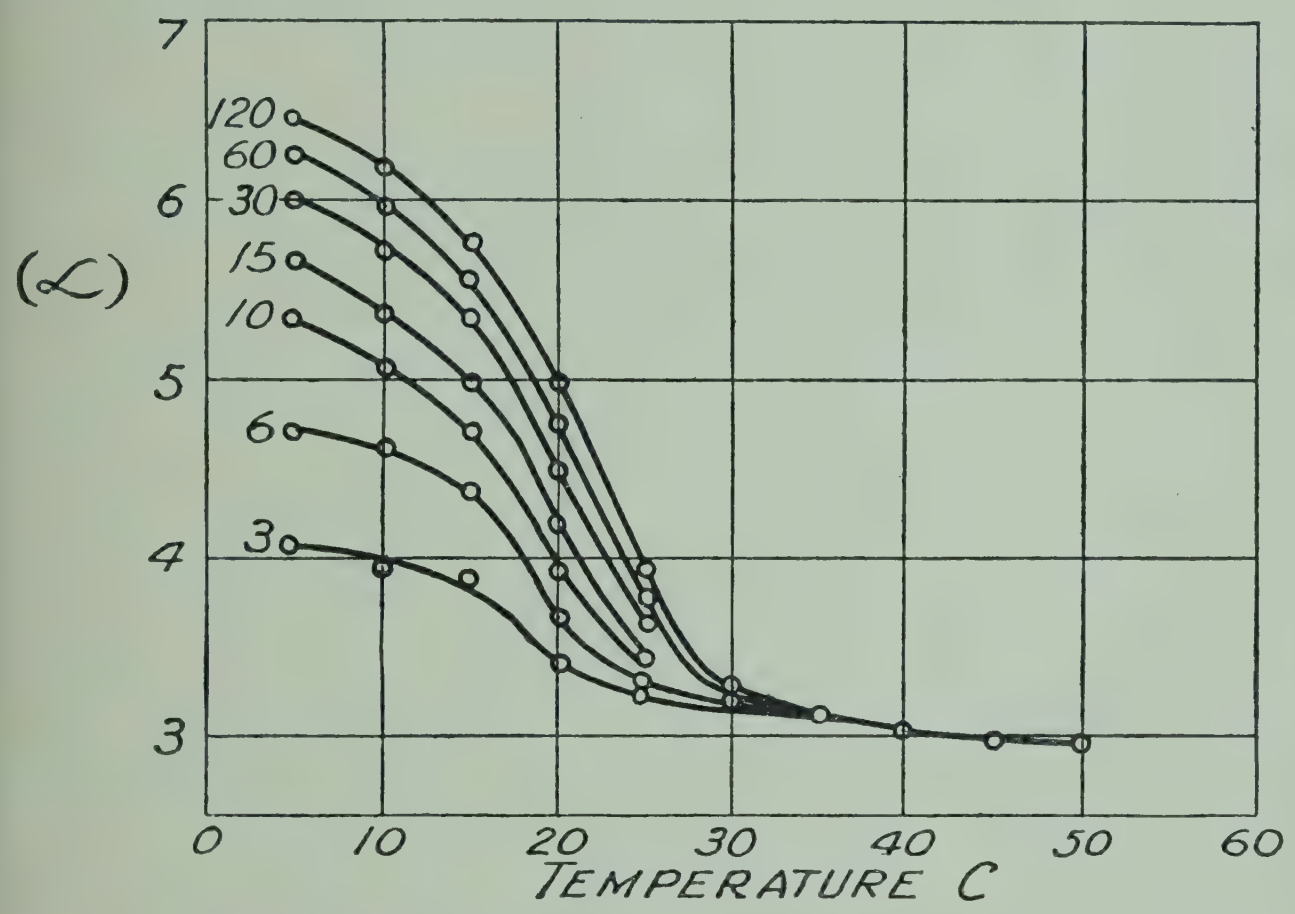


FIG. 13.—Convergence of the temperature—specific rotation curves of gelatin. Ordinates refer to rotation.

maximum at about pH 8. At low temperatures there is a region of high specific rotation at pH 1—1·5, and even at higher temperatures distinct irregularities are apparent in such low pH curves.

Extending this investigation [1] it was concluded that the optical activity of gelatin is a function of such colloid features as degree and state of dispersion, in addition to the intramolecular stereochemical relations. At temperatures greater than 30°—40° C., gelatin hydrosols behave as ordinary solutions, and with change in pH value or temperature there is little alteration in light-scattering property, apparent viscosity or optical activity. The last two factors have a poorly defined minimum value at about pH 5. It is possible that a minimum is existent at about pH 1 and pH 8·5.

[1] Kraemer and Fanselow, *J. Phys. Chem.*, **32**, 894 (1928).

Reduction in temperature gives rise to new phenomena. Regions of precipitation [1] at various pH values, especially close to pH 5 and pH 1, are revealed by the Tyndall effect. In these regions in the case of dilute gelatin solutions optical rotation and viscosity do not exhibit their maximum values and the gelation tendency is poor. However, immediately adjacent to these precipitation regions there exist narrow regions, where the gelation tendency is very marked, and the optical rotation shows a pronounced increase. There is too, a steep rise in the resistance to shear, but no longer independent of the rate of shear. "In fact, the behaviour with respect to deformation is that of a weak elastic solid, which is broken by passage through a capillary tube." The capacity to scatter light is very low, and such factors as pH, temperature, electrolytes or concentration of gelatin which prevent gelation, also prevent the characteristic rise in optical rotation.[2]

Kraemer and Fanselow point out how significant is the simultaneous appearance of gel formation, high specific rotation and low Tyndall effect in such close proximity to a region of precipitation. The incipient or incomplete precipitation is considered as first evidence of gel tendency, and although the optical rotatory power is greatly modified, the optical heterogeneity (Tyndall cone) is not appreciably affected. "The rigidity and elasticity characteristic of the gel state may arise without the appearance of the grosser features associated with advanced stages of precipitation."

Since the optical rotation is so intimately related to colloidal features, it militates against the conception that mutarotation and gel formation in gelatin solutions involve tautomerism of the sol form $\rightleftharpoons$ gel form. It seems highly probable that the fundamental cause of the rotatory behaviour is due to structural features inherent in the gel state and on a supermolecular scale.

Experiments were conducted with a de-ashed hide gelatin, electrodialysed to the isoelectric point. The ash content was 0·05 per cent. To avoid disturbances due to thermal history, all solutions were maintained at 50° C. for 20 minutes.

The general conclusions reached by the authors were :—

(1) The specific rotation in the case of solutions above 30° C. is almost independent of pH, temperature, specific electrolyte, and concentration of gelatin, over somewhat wide limits for these factors. More or less clearly defined minima were noted at pH values near 1, 5, and 8·5, the falls in rotation being rapid at the two extremes.

(2) Maximum values of specific rotation were observed near pH values 1, 5, and 8·5 when the temperature was below 25° C., and were particularly prominent with low gelatin concentrations and low

[1] Kraemer and Dexter, *ibid.*, **31,** 764 (1927).
[2] *Vide* Carpenter, *ibid.*, **31,** 1873 (1927).

temperatures. Different electrolytes exert a small lyotropic effect. Within this temperature range mutarotation is seen, *i.e.*, "the rotatory power adjusts itself with measurable speed to change in temperature."

(3) A striking parallelism exists between the optical behaviour and the colloidal behaviour, especially the power to form gels. In dilute solutions gel formation is always accompanied by marked mutarotation and a high specific rotation. Such factor as prevents setting to a gel will also cause a low specific rotation.

Fanselow [1] followed up this research and confirmed Kraemer's two types of temperature—specific rotation curves. At the higher temperatures there is marked dispersion of the gelatin micellæ and the independent molecules are not attached. At lower temperatures the specific rotation becomes a function also of the concentration of the gelatin. There is now intermicellar attraction between the gelatin micellæ thus providing higher resistance to shear and increased optical activity.

Fanselow investigated the influence of the nature of the acid employed on the optical activity of a gelatin solution at 15° C. over the pH range 0·5—5. Between the isoelectric point and pH 3 the curves for specific rotation and resistance to shear were independent of the nature of the acid, but at low pH values there are wide differences according to the organic or inorganic class. Even where organic acids permit such a low pH value as 0·5, there is no increase in specific rotation and relative resistance to shear at 15° C. in the region pH 1·2. Partially decreasing the hydration by means of acetone emphasises the results. Apparently, the influence of the acid does not depend on the valence of the anion; rather is there a Hofmeister ion effect.

The quantitative results of the work of Kraemer and Fanselow are confirmed by Bogue and O'Connell [2] and Carpenter, Dahlberg and Hening.[3] The last-named authors propose the grading of gelatin samples for gel capacity by the mutarotation method *at a definite pH value*, suggesting pH 7·3 for calfskin and bone gelatins, and pH 5 for pigskin gelatins. The necessity for accurate thermal control of the solutions is emphasised by Reiger [4] who showed that if a gelatin solution is gelatinised at low temperature and is then brought to a higher temperature, the rotation decreases.

THE GEL STRENGTH OF GELATIN

The strength of gelatin jelly preparations has obvious importance in food technology, although most of the earlier work [5] done on gel

[1] *Am. Coll. Symp. Monog.*, **6,** 237 (1928).
[2] *J. Am. Chem. Soc.*, **47,** 1694 (1925).
[3] *Ind. Eng. Chem.*, **20,** 397 (1928).
[4] *Kolloidchem. Beihefte*, **19,** 412 (1924).
[5] See Lipowitz, "Neue Chem.-tech. Untersuchungen" (Berlin, 1861), p. 37;

strength was connected with the testing of glue. Inquiries concerning gel strength demand consideration of such factors as pH, temperature, concentration, thermal history and so on.

Various pH values corresponding to maximum gel strength have been reported. Thus, Oakes and Davis,[1] measuring the gel strength of a 5 per cent. gel of hide gelatin at 21° C. over the pH range 1·3—13, give a maximum at pH 8. Sheppard and Sweet [2] report maxima at pH 4·6, pH 5·0 and pH 6·6. Carpenter, Dahlberg and Hening [3] employed de-ashed calfskin gelatin of concentration 6·67 per cent., and gels were " hardened " at 10° C. and 0·7° C. respectively. Gel strength was at a maximum at about pH 6·0, the graph being unsymmetrical about this point as axis. For practical purposes, one can speak of a zone of maximum gel strength, pH 5—7.[4] The colder gel (0·7° C.) showed about 65 per cent. increased gel strength in the maximum zone.

Kraemer [5] has discussed the problem from the axiom that gelation is due to incomplete or unsuccessful precipitation of the disperse phase from the colloidal solution. He found maximum gel strength to be associated with gelatin on either side and very close to the isoelectric point (optimum precipitation point). At low temperatures, another region of pronounced gel strength was observed at pH 1—1·5, associated with a maximum Tyndall effect.

According to Freundlich and Seifriz, [6] the elasticity of the gel, increases rapidly and uniformly as the concentration increases, reaching a maximum at 0·8 per cent. gelatin. The grade of the gelatin has, of course, a profound influence.

A jelly-strength testing machine operating by torsion, was used by Sheppard, Sweet and Scott [7] to twist a cast cylinder of jelly at a uniform rate. After forming a jelly at 0° C. for 3 hours, it was observed that subsequent elevation of temperature up to 10° C. had no appreciable effect on the gel strength, but higher temperatures resulted in a rapid decrease in gel strength. No simple relation was found to hold between concentration and gel strength and the curves connecting these data for different grades of gelatin do not belong to a common family and frequently intersect.

Regarding the temperature factor, Tracey [8] observed an almost

Kieser, *Kolloid-Z.*, **28,** 186 (1921); Kissling, *Chem. Ztg.*, **17,** 726 (1893); **22,** 171 (1898); Valenta, *ibid.*, **33,** 94 (1909); Scott, *Chem. Eng.*, **5,** 441 (1907); Alexander, *J. Soc. Chem. Ind.*, **25,** 158 (1906); Hulbert, *Ind. Eng. Chem.*, **5,** 235 (1913); Low, *ibid.*, **12,** 355 (1920); Smith, U.S. Pat. 911, 277 (1909); Smith, *Ind. Eng. Chem.*, **12,** 878 (1920); Sheppard *et al.*, *ibid.*, **12,** 1007 (1920), and *J. Am. Chem. Soc.*, **43,** 539 (1921); **44,** 1857 (1922); Oakes and Davis, *Ind. Eng. Chem.*, **14,** 706 (1922).

[1] *Loc. cit.*
[2] *J. Am. Chem. Soc.*, **43,** 539 (1921).
[3] *Ind. Eng. Chem.*, **20,** 397 (1928).
[4] Cf. Sheppard and Sweet, *ibid.*, **16,** 595 (1924).
[5] *Am. Coll. Symp. Monog.*, **4,** 110 (1926).
[6] *Z. physik. Chem.*, **104,** 233 (1923).
[7] *Ind. Eng. Chem.*, **12,** 1007 (1920).
[8] *J. Soc. Chem. Ind.*, **47,** 94 T (1928).

horizontal inflexion in his curves in the range 19°—23° C. The following are comparative gel strengths in relation to temperature : 1·0, 4·4, 5·9, 9·1, 9·8, and 14·6 for temperatures 27° C., 24° C., 21° C., 18° C., 15° C. and 12° C. respectively.

Interesting results have been published by Carpenter, Dahlberg and Hening [1] who used de-ashed calfskin gelatin.

The influence of concentration and temperature is given by the authors in Table 17 for systems at pH 6·0.

TABLE 17. EFFECT OF GELATIN CONCENTRATION AND TEMPERATURE ON BLOOM STRENGTH AT pH 6·0.

No.	Dry Gelatin.	Bloom Strength.			
		10° C.		0·7° C.	
		18 Hours.	42 Hours.	18 Hours.	42 Hours.
	Per Cent.	Grams.	Grams.	Grams.	Grams.
1	6·85	376	415	578	610
2	6·67	354	—	558	—
3	6·00	314	346	479	515
4	5·00	224	248	356	384
5	4·00	150	165	241	259
6	2·95	87	92	138	148
7	2·00	41	42	69	72
8	1·00	(too weak to measure)		23	24

Discussing their curves, the authors note that at 0·7° C. the relation between gelatin concentration and gel strength for a given gelatin is a simple parabola and over a considerable range of concentration is practically linear. At 10° C., however, the parabolic curve no longer holds, the values forming a smooth curve in each case. It is noted, also, that the Bloom instrument really measures gel elasticity.

The extraordinary sensitivity of gelatin to heat is reflected in gel strength ; the time and temperature during gelation are most important. The authors' data show that in the case of higher gelatin concentrations, setting to a gel is more complete in the first 18 hours at 0·7° C. than at 10° C., but in neither case is gelation fully accomplished even after 42 hours. In fact, with dilute solutions of gelatin, two weeks are necessary for gelation to be completed.

Bearing on this problem are some results by Neville, Theis and Oswald,[2] who studied the repeated alternate gelation and melting of a 10 per cent. gelatin solution. Maintaining the gel condition for 48 hours increased the hysteresis by about 10° C. Repeated melting

[1] *Ind. Eng. Chem.*, **20,** 397 (1928).
[2] *Ibid.*, **22,** 62 (1930).

and gelation decrease the hysteresis, *i.e.*, brings the melting-point and setting-point into closer proximity.

Again, Kraemer [1] has reported interesting experiments showing the influence of the thermal history of a gelatin sol on its gel strength between pH 4·4—4·8. Under these conditions the strength of a gel formed at 15° C. is greater than that formed at 20° C. When, however, the total history is split, so that the gel is kept for one period at 15° C. and for another period at 20° C., the results vary, thus : (*a*) if the lower temperature precedes the higher, the gel strength is less than that, given by a gel, which was set continuously at 20° C. ; (*b*) if the higher temperature period precedes the lower, the gel strength is greater than that of a gel, which was set continuously at 15° C.

The actual laboratory methods employed for the determination of the gel strength of gelatin are beyond the scope of this volume, but the student is referred to accounts of the standard Bloom test, using the Bloom gelometer.[2] Other methods are described by Briefer and Cohen,[3] Tracey,[4] Smith,[5] and Oakes and Davis.[6]

[1] *Am. Coll. Symp. Monog.*, **4,** 120 (1926).
[2] *Ind. Eng. Chem.* (*Anal. Edn.*), **2,** 349 (1930) ; *Ind. Eng. Chem.*, **16,** 311 (1924) ; *ibid.*, **21,** 264 (1929) ; Richardson, *Chem. Met. Eng.*, **28,** 551 (1923).
[3] *Ind. Eng. Chem.*, **20,** 408 (1928).
[4] *J. Soc. Chem. Ind.*, **47,** 94 T (1928).
[5] *Ind. Eng. Chem.*, **12,** 881 (1920).
[6] *Ibid.*, **14,** 706 (1922).

CHAPTER V

COLLOIDS IN CEREAL CHEMISTRY

THE COLLOID CHEMISTRY OF THE STARCHES

The food chemist is concerned with the physico-chemical behaviour of starch in several foods, but particularly so in the study of bread. The present section will briefly summarise our knowledge of the colloid chemistry of various starches, a later section discussing in more detail its *rôle* in bread-making.

Starch is an important constituent of such cereals as wheat, rice, maize, etc., and vegetables such as potatoes. Thus, wheat flour contains 65—70 per cent. of starch, maize 60—65 per cent., chestnuts 42 per cent., potato 18 per cent., lentils 57 per cent., and rice 79 per cent. The chemical constitution of a starch is still a debatable question among organic chemists, and its discussion is outside the scope of the present volume. According to Ling,[1] "the general conclusions arrived at regarding the constitution of all starches is that they contain two basal units, α-hexa-amylose and αβ-hexa-amylose. These exist in starch granules in a form polymerised possibly to different degrees in the different starches, by subsidiary valencies and esterified. They then occur as polymerised α-amylose and amylopectin. In addition to these two substances some starches, notably those of the cereals, contain a third substance amylohemicellulose."

Irvine [2] points out that starch is not a definite chemical individual, but that the term refers "to a variety of closely-related compounds which share the common property of conversion, by standard processes, into glucose or maltose respectively, and to each of which the empirical formula $(C_6H_{10}O_5)x$ may be applied." [3] The degree to which inorganic constituents are combined in the starch differs with different starches, as does also the degree of polymerisation. Pictet [4] believes that the simplest molecule of "soluble" starch is $(C_6H_{10}O_5)_{18}$.

[1] Walton's "A Comprehensive Survey of Starch Chemistry," Vol. 1 (New York, 1928), p. 33.

[2] *Ibid.*, p. 16.

[3] *Vide*, Chodat, *Compt. rend, des séances soc. phys. hist. nat. Geneva*, **41,** 122 (1920); Ling, *J. Soc. Chem. Ind.*, **46,** 279 T (1927); Philia, *Bull. soc. botan. Geneva*, (2), **16,** 519 (1924); Kuhn, *Ann.*, **443,** 1 (1925); Windisch, *Wochschr. Brau.*, **41,** 3 (1924); **42,** 181, 187, 193, 199 (1925); **44,** 369 (1927); Irvine and MacDonald, *J. Chem. Soc.*, **129,** 1502 (1926); Peiser, *Z. physiol Chem.*, **167,** 88 (1927); 161, 210 (1926); Sponsler, *J. Gen. Physiol.*, **5,** 757 (1923); Karrer, *Naturwissenschaften*, **9,** 399 (1921); Weidenhagen, *Z. Ver. deut. Zucker—Ind.*, **77,** 474 (1927).

[4] *Helv. Chim. Acta.*, **9,** 33 (1926). See also an important paper on "The Constitution of Starch," by Meyer, Hopff, and Mark, *Ber.*, **62 B,** 1103 (1929); also "Theory of Starch Formation," by Josephson, *Z. physiol. Chem.*, **174,** 179 (1928). An

STARCH GRANULES

Starch granules show typical concentric rings and their appearance under the microscope serves in analytical differentiation.[1] Schade [2] points out that these rings are not due to a Liesegang effect but to appositional growth. "The dark-granuled chlorophyll containing plasts, are distinctly visible as they build up the separate starch granules at the poles of the masses. These chloroplasts add layer on layer of new starch-colloid." Under polarised light the granules show a dark cross, evidence of stress in the layers caused by shrinkage with age. The cross is lost on subsequent swelling in water.[3]

Nowopokrowsky and Tschebotarewa [4] and Amar [5] hold the view that the intact starch granules probably consist of strongly dehydrated and compressed polysaccharides, which form layers of differing degrees of hydration. Only in the heart of the granule is there possibly a colloidal solution of amylose. The granules vary in size,[6] *e.g.*, the diameter in mm. is given by Lloyd [7] as 0·004, 0·0142 and 0·040 for rice, maize and potato starch respectively.

FRACTIONATION OF STARCH

As early as 1825, Raspeuil [8] recognised the heterogeneous character of starch granules, having observed both soluble and insoluble components which gave different colours with iodine.

Meyer [9] and Maquenne [10] investigated the fractionation of starch and their different nomenclatures have to some extent confused the subsequent literature. Thus :—

Meyer	starch granules	outer portion	= α-amylose.
		inner ,,	= β-amylose.
Maquenne	starch granules	outer ,,	= amylopectin.
		inner ,,	= amylose.

excellent historical survey is given by Samec, "Kolloidchemie der Stärke" (Dresden and Leipzig, 1927), 1—94, and by Eynon and Lane, "Starch" (Cambridge, 1928), 16—44.

[1] Cf. Allen, *Philippine J. Sci.*, **38,** 241 (1929); Reichert, Carnegie Institution of Washington, *Pub.*, **173** (1913); Allen's "Commercial Organic Analyis" (London, 1924), p. 514 *seq.*; Greenish, "The Microscopical Examination of Foods and Drugs" (London, 1923), 1—23.

[2] Alexander's "Colloid Chemistry" (New York, 1928), Vol. 2, 834.

[3] Meyer, "Untersuchungen über die Stärkekörner" (Jena, 1895). See also, Samec, "Kolloidchemie der Stärke" (Dresden and Leipzig, 1927), p. 82 *seq.* on "Der Aufbau des Stärkekörnes."

[4] *Kolloid-Z.*, **52,** 304 (1930).

[5] *Compt. rend.*, **178,** 1317 (1924).

[6] *Vide* Samec, *loc. cit.*, p. 94.

[7] *J. Am. Chem. Soc.*, **33,** 1213 (1911).

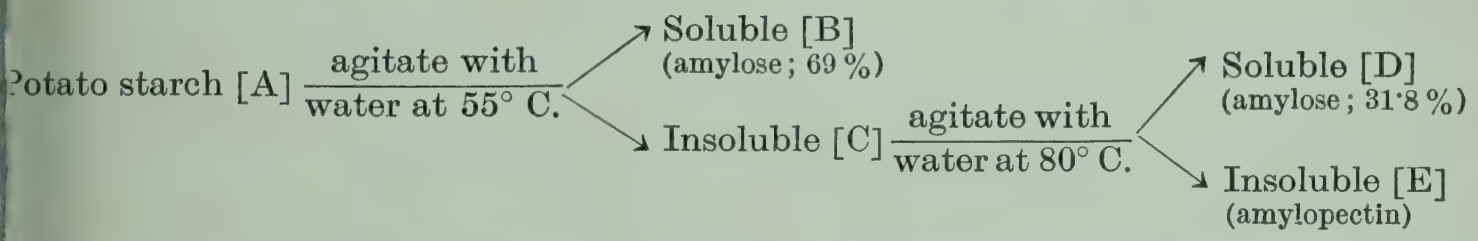
[8] *Ann. sciences naturelles*, **6,** 224, 384 (1825).

[9] *Loc. cit.* This nomenclature has been revived by Taylor, Walton's "A Comprehensive Survey of Starch Chemistry" (New York, 1928), Vol. I., p. 62; *Ind. Eng. Chem.*, **18,** 713 (1926); *J. Am. Chem. Soc.*, **48,** 1739 (1926); **51,** 294 (1929).

[10] *Ann. chim. phys.*, **2,** 109 (1904); **9,** 179 (1906).

Ling and Nanji [1] have observed that amylopectin is associated with amylose within the starch granule, only about one half being extractable with dilute alkalies. In all starch granules they found the ratio of amylose to amylopectin as 2 : 1. This conclusion must be modified in the light of Schryver's observation that certain starch granules (rice, maize, barley, wheat, etc.) contain also a hemicellulose (absent in potato or arrowroot starch). According to Ling and Nanji, "hemicellulose" is really a Ca—Mg salt of a silicic and phosphoric ester of α-amylose, *i.e.*, it is an amylohemicellulose. It is probably the encrusting substance of the granule. Fargher and Probert [2] found the following approximate percentages of hemicellulose in wheat, maize and rice starch : 23, 21 and 12 respectively. Amylopectin is generally considered to be a complex of phosphoric acid and carbohydrate.[3]

Numerous investigations have been made relating to the problem of separating amylose and amylopectin from starches. Simple treatment with hot water can effect the fractionation of starch into the water-soluble amylose and the insoluble amylopectin. Thus, Hess, Friese and Smith [4] obtained 78·8 per cent. amylose and 21·2 per cent. amylopectin from potato starch, their procedure being as follows :—



Examination under the microscope showed that the outer shell of the starch granule was made up of (B) and (D) and the nucleus of (E) this constituent, amylopectin, containing 0·209 per cent. of P_2O_5.

The recent work of Nowopokrowsky and Tschebotarewa [5] led them to conclude that a paste of potato starch (1 per cent. suspension in water) is really a suspension of amylopectin "sheaths" in a colloidal solution of amylose. The amylose solution filters through ordinary filter paper giving a transparent liquid from which the colloidal amylose can be separated by the use of collodion membranes, or precipitated by addition of $MgSO_4$, K_2SO_4 or $(NH_4)_2SO_4$. The amylose solution shows typical Brownian motion when seen under the ultramicroscope.[6]

Ling and Nanji [7] separate the starch constituents by holding the

[1] *J. Soc. Chem. Ind.*, **46,** 279 T (1927). See also, *J. Inst. Brewing*, **30,** 764 (1924) and Samec and von Hoefft, *Kolloidchem. Beihefte*, **3,** 141 (1913).

[2] *J. Textile Inst.*, **18,** 571 T (1927).

[3] *Vide*, Malfitano and Cattoire, *Ann. Brass. Dist.*, **20,** 291 (1922) ; *Kolloid-Z.*, **46,** 3 (1928) ; Polak and Tychowski, *Biochem. Z.*, **214,** 216 (1929) ; Samec, *Kolloidchem. Beihefte*, **8,** 33 (1916) ; *Kolloid-Z.*, **47,** 81 (1929), and in Walton's "A Comprehensive Survey of Starch Chemistry" (New York, 1928), p. 51.

[4] *Ber.*, **61 B,** 1975 (1928).

[5] *Kolloid-Z.*, **52,** 302 (1930).

[6] Cf. Agazzotti, *Z. allgem. Physiol.*, **7,** 862 (1907).

[7] *J. Chem. Soc.*, **123,** 2666 (1923) ; Brit. Pat., 217,770 (1924).

starch paste at 0° C. for 10—12 hours, whereby the starch is precipitated as fibrous masses. On now holding the mass below the gelatinising temperature, amylose goes into solution, leaving behind the insoluble amylopectin. The presence of 0·5—1 per cent. Na_2CO_3 aids in the more effective removal of amylose. Alternatively, starch paste is treated with barley diastase at 50° C., whereby there is complete conversion of amylose to maltose, the amylopectin remaining practically untouched.

By centrifuging a 1 per cent. starch paste, a closer fractionation is permitted.[1] Various more complicated methods [2] are also employed, such as Gatin-Gruzewska's method of treating 3 per cent. starch paste with one quarter its volume of 40 per cent. NaOH at 60° C. and then adding one-third of the total volume of 95 per cent. alcohol, thus precipitating the amylopectin.[3]

The following table collects the more important quantitative findings of the different investigators :—

TABLE 18. FRACTIONATION OF STARCHES.

Starch.	Amylopectin.	Amylose.	Observer.
Barley.	31·8 per cent.	—	Ling and Nanji.[4]
Maize	11·5—15·6	84·4—88·5	Taylor and Iddles.[5]
,,	44·7	—	Samec.[6]
,,	70·0	—	Tanret.[7]
,,	—	5	Fargher and Probert.[8]
Potato.	55—60	40—45	Gatin-Gruzewsha.[3]
,,	—	27	Tanret.[7]
,,	83	17	Samec and Mayer.[9]
,,	—	17	Fargher and Probert.[8]
,,	85	15	Sherman and Baker.[10]
,,	21·44	15·50	Baldwin.[11]
Rice.	—	12	Fargher and Probert.[8]
,,	68·5	—	Tanret.[7]
,,	38·2	—	Samec.[6]
Tapioca.	16·3—17·5	—	Taylor and Walton.[12]
Wheat.	23·5—23·8	—	Taylor and Walton.[12]
,,	67·5	—	Tanret.[7]
,,	33·7	—	Ling and Nanji.[4]
,,	—	13	Fargher and Probert.[8]

[1] Karrer and von Krauss, *Helv. Chim. Acta.*, **12,** 1144 (1929) ; Sherman and Baker, *J. Am. Chem. Soc.*, **38,** 1885 (1916) who use dil. NaCl, *e.g.*, a 2 per cent. starch paste + 0·002 M. NaCl, heated to 75° C.

[2] Cf. Samec " Kolloidchemie der Stärke " (Dresden and Leipzig, 1927), p. 13 *seq.*

[3] *Compt. rend.*, **146,** 540 (1908) ; **152,** 785 (1911).

[4] *J. Chem. Soc.*, **123,** 2666 (1923) ; **127,** 629 (1925).

[5] *Ind. Eng. Chem.*, **18,** 713 (1926).

[6] *Loc. cit.*, p. 194.

[7] *Bull. soc. chim.*, **17,** 83 (1915).

[8] *J. Textile Inst.*, **18,** 569 T (1927).

[9] *Kolloidchem. Beihefte*, **13,** 272 (1921).

[10] *J. Am. Chem. Soc.*, **38,** 1885 (1916).

[11] *Ibid.*, **52,** 2907 (1930).

[12] *Ibid.*, **51,** 3431 (1929).

RECENT INVESTIGATIONS ON FRACTIONATION

Two important communications have recently thrown considerable light on the question of the fractionation of starch. Taylor and Walton [1] had two aims in their investigation : (1) to characterise a starch as a function of its constituent amyloses, (2) rigidly to define the amyloses by their chemical properties.

Starch was treated with alcoholic HCl at 50° C. and subsequently gelatinised by NH_4CNS. The starch was then precipitated by alcohol and dried. A suspension in water was now subjected to electrodialysis for 1—2 weeks, whereby there occurred a sharp separation of solid at the anode, leaving a clear upper liquor containing amylose, which could be thrown out of solution by alcohol. The properties of the two fractions so obtained from starches prepared from corn, rice, potato, wheat and tapioca were : (*a*) *amylopectin*—insoluble in water ; retained by collodion membranes, which would pass amylose ; migrated to the anode ; (*b*) *amylose*—fairly soluble in water ; exhibited no electro-migration. The ratio of amylopectin to amylose was 17 : 83 for starches from tapioca, corn and rice, and 24 : 76 for wheat starch.

A comprehensive investigation by Baldwin [2] concerned potato starch and had similar aims to the above. Amylose was separated by first gelatinising at 1·5 per cent. starch paste at 63° C. and then freezing it. The frozen mass was then melted at 40° C. and filtered through filter paper (Filtrate I.). The residue was again heated in water at 55°—60° C. for 16 hours and filtered. This operation was repeated, the residue frozen and the extraction at 50°—60° C. repeated four times. The six extracts contained the amylose, obtainable in solid form by precipitation with alcohol. The amylopectin was obtained by heating a 1·5 per cent. starch paste to 85° C. for 2 hours and then freezing. The frozen mass was melted and filtered, the residue suspended in excess of distilled water at room temperature, and after decantation, repeating the operation at room temperature, then at 85° C. and again three times at room tempera-

	Amylose.	Amylopectin.
Specific Optical Rotation $[\alpha]_D^{20}$.	189°	195·5°
Temperature coefficient $[\alpha]_D^{20}$.	0·13	1·23
Phosphorus content . .	0·0009 per cent.	0·076 per cent.
Colour with iodine . .	Blue.	Red-violet.
Precipitation by alcohol . .	98–99 per cent.	No precipitate in absence of electrolyte.
Precipitation by iodine . .	99 per cent.	No precipitate.
Retrogradation . . .	90–95 per cent.	*Nil.*

[1] *Ibid*, **51,** 3431 (1929).

[2] *J. Am. Chem. Soc.*, **52,** 2907 (1930); Cf. *Ind. Eng. Chem.* **18**, 713 (1926) ; Samec, *loc. cit.*, p. 192 ; Freundlich and Nitze, *Kolloid-Z.*, **41,** 209 (1927).

ture. The grain envelopes, amylopectin, may be suspended in water and kept under toluene, or may be dried and powdered.

Baldwin's investigation of the properties of amylose and amylopectin were summarised by him (see Table on p. 119).

THE PREPARATION OF STARCH

Numerous authors have described the commercial preparation of starches.[1] As example, Williams [2] outlines the preparation of rice starch as follows : Rice is steeped in cold, dilute NaOH (S.G. 1·005) for 24 hours, and then the alkali is replaced by a fresh lot. The steeping is continued for 36—48 hours, until the grain softens and forms a homogeneous mass. The whole is now disintegrated mechanically at a temperature not exceeding 30° C. Sedimentation in dilute alkali is permitted at a temperature below 15° C. for 12—14 days, whereby the impure starch comes to the top as a plastic, semi-solid mass. This is removed and thoroughly mixed with water for an hour and then permitted to settle for 12 hours. The effluent is run off and water added to make a starch suspension of S.G. 1·210. This is filtered and the starch dried at about 57° C. Such rice starch contains as a rule, 17·8 per cent. moisture and 0·6 per cent. impurities.

Wheat starch figures prominently in cereal chemistry and, therefore, its preparation for research work is of interest.[3] About 100—125 gr. of wheat flour are mixed with tap water to make a stiff dough which is then kept immersed in water for about 45 minutes to ripen the gluten. Subsequent kneading of the dough under a gentle stream of water serves to remove starch, which passes into suspension in the water (1 litre). Addition of NaCl to 1 per cent. concentration serves to dissolve globulins. The starch is washed with water until chloride-free, using a centrifuge to render the process more rapid and more complete. After three washings with 95 per cent. alcohol, and four washings with anhydrous ether, the starch is dried *in vacuo* for 5—7 days and then stored in a dessicator over $CaCl_2$. The moisture content varies from 2·28—5 per cent. and the nitrogen content from 0·031—0·047 per cent. Alternatively, wheat flour may be subjected to enzyme action to remove proteins.[4]

PROPERTIES OF STARCH

The following figures by Fargher and Probert [5] indicate the order of purity of a commercial Japanese farina, and high grade

[1] See Moffett on Corn Starch in Walton's " A Comprehensive Survey of Starch Chemistry," p. 130 ; also Preuss, *ibid.*, p. 139, on Potato Starch ; Auden, " Starch and Starch Products " (London, 1922).

[2] *Ind. Chem.*, **6,** 387 (1930).

[3] Rask and Alsberg, *Cereal Chem.*, **1,** 12 (1924).

[4] Campbell, Brit. Pat., 182,829 (1923).

[5] *J. Textile Inst.*, **18,** 561 T (1927).

potato, wheat, maize and rice starches. The percentage results are calculated on the basis of dry starch :—

	Glucose-yielding Carbohydrates.	Ash.	P_2O_5.
Japanese farina . . .	94·0	0·28	0·148
Potato starch . . .	97·6	0·33	0·167
Wheat starch . . .	96·6	0·23	0·132
Maize starch . . .	97·5	0·20	0·049
Rice starch. . . .	96·1	0·67	0·189

According to Preuss [1] a good, high-grade potato starch should have the following approximate analysis : Pure starch = 99·18 per cent., protein = 0·28 per cent., fibre and fat = 0·30 per cent., and ash = 0·24 per cent. The moisture content of potato starch varies from 18·9—22·6 per cent.[2]

When pure, starch is a white solid possessing neither taste nor smell. In the dry state it is stable in air up to about 105° C. On heating to 150°—160° C. it dextrinises and becomes slightly yellow in colour and is rendered water soluble. Although commercial starches are acid (and occasionally alkaline) in reaction, pure starch is neutral in reaction. It is insoluble in organic solvents and it gives no precipitate with Fehling's solution.

THE SYSTEM : STARCH/WATER

Dry starch granules swell in cold water,[3] mechanical disruption of the granules assisting such swelling.[4] By dry-grinding starch and then mixing in *cold* water Alsberg showed that over 60 per cent. in certain samples passed into colloidal solution, and under the microscope starch granules were readily recognisable.[5] Cold opalescent starch solutions were thus prepared, containing nearly 10 per cent. of starch, the solutions being markedly viscous and increasing in viscosity on standing. Further, optically clear 0·4 per cent. starch solutions were prepared in cold water, the solution giving an intense blue colour with iodine, and dialysing through thin collodion membranes.

On heating with water, starch swells [6] more intensively and viscous pastes result. In taking up water, starch swells with

[1] *Loc. cit.*, p. 155.
[2] Vogelenzang, *Pharm. Weekblad.*, **64,** 1069 (1927).
[3] Meyer, " Die Stärkekörner " (Jena, 1895), p. 127.
[4] Alsberg, *Ind. Eng. Chem.*, **18,** 191 (1926). See also, Jessen, *Ann. Phys. Chem.* (*Poggendorff's*), **106,** 497 (1859), and Flueckiger, *Z. Chem. Pharm.*, **4,** 104 (1861).
[5] Cf. Taylor and Beckmann, *J. Am. Chem. Soc.*, **51,** 299 (1929).
[6] Nowopokrowsky and Tschebotarewa, *Kolloid-Z.*, **52,** 304 (1930).

evolution of heat. This has been quantitatively investigated by Rodewald [1] who observed that the original moisture content of the starch determined the heat of swelling. If the initial moisture content exceeds a certain value (characteristic for different starches), the heat of swelling is *nil*. Thus, the limiting values of moisture for arrowroot, potato, rice and wheat starch are : 29·99 per cent., 37·10 per cent., 26·79 per cent., and 31·63 per cent. respectively. Most starches swell but do not burst in boiling water, potato starch being a notable exception, inasmuch as it disintegrates and yields a colloidal solution in which are suspended fairly coarse fragments of starch granules.[2] In general, starch solutions after boiling, contain clear granules filled with fluid.

Katz [3] points out that he has confirmed important observations reported by Meyer [4] and by Beijerinck,[5] who found that the gelatinisation of starch granules in water causes the starch granules to swell " much more strongly in the tangential direction than in the radial direction. A cavity is formed in the centre of the starch granules and this increases gradually in size until finally the starch granule is changed to a many times larger, tightly filled vesicle or bladder." Gelatinised starch constitutes the vesicle walls, whilst the contents of the vesicles consist of an aqueous starch solution.

Gelatinisation. The gelatinisation of various starches upon heating in water has been studied by many investigators, but the results obtained are far from being concordant. Katz [6] believes that swelling or gelatinisation of wheat starch in water is an equilibrium process, the limiting condition depending upon the temperature and the relative amount of water present. Sallinger [7] concluded that the starch is present both as a sol and a gel, the former protecting the latter, a view somewhat allied to that of Biedermann, who believed the hydrogel to be of amylopectin.[8] Similarly, Hess [9] investigated the fractional solubility at 80° C. of potato starch in relation to amylose and amylopectin contents. Lepeshkin [10] studied the problem of the high temperature coefficients of the swelling of starch in hot water and decided that a chemical union of water and starch took place, whilst Malfitano and Catoire [11] concluded that the alkaline earth salts of phosphoric and silicic acids account for the degree of solubility of the starchy material.

[1] *Z. physik. Chem.*, **24,** 193 (1897) ; **33,** 579 (1900).
[2] Harrison, *J. Soc. Dyers Colourists*, **27,** 84 (1911).
[3] Walton's " A Comprehensive Survey of Starch Chemistry," p. 112.
[4] *Loc. cit.*, 129—136.
[5] *Proc. Amsterdam Akad. Wetens.*, **14,** 1107 (1912).
[6] *Verslag. Akad. Wetenschappen.*, **23,** 1150 (1915).
[7] *Kolloid-Z.*, **25,** 79 (1919).
[8] *Arch. ges. Physiol.*, **183,** 168 (1920).
[9] *Ber.* **61 B.**, 1975 (1928).
[10] *Bull. soc. bot. Geneva*, **13,** 40 (1921).
[11] *Compt. rend.*, **177,** 1309 (1923).

The following table collects some of the published data [1] relating to the gelatinising temperatures of starches in water :—

Starch.	Gelatinising Temperature, °C.	Observer.
Arrowroot . . .	74·6	Francis and Smith.
Cassava	69—70	Harvey.
Corn	71—72	Harvey.
,, (13 varieties) . .	64·1—71·1	Dox and Roark.
,, ...	70·9	Francis and Smith.
Maize	66—68	Levites.
,, ...	70	Stocks.
Potato	66—67	Harvey.
,, ...	63	Stocks ; Baldwin.
,, (sweet) . . .	82·5	Francis and Smith.
,, (Irish) . . .	67·7	,, ,,
Rice	74	Stocks.
Rye	70—75	Herter.
Sago	72	Stocks.
Wheat	62·2	Francis and Smith.
,, ...	65	Stocks.
,, ...	70	Kent-Jones.
,, ...	>75	Herter.

A critical discussion of the phenomena of the swelling of starches in hot water has been presented by Alsberg [2] who points out that three factors are involved :—

(1) The rigidity of the granules, *i.e.*, the extent to which the anatomical structure is softened by moist heat.

(2) The actual capacity of the granules to absorb water.

(3) The ratio : $\frac{\text{mass of swelling substance.}}{\text{surface area of the granule.}}$

The latter factor infers that larger granules should swell more readily than smaller ones, and in fact, Whymper observed that the larger granules of starches gelatinise at a lower temperature than the smaller ones.[3] That grain-size is important in this connection was also proved by Rosenthaler [4] in his study of the swelling in KOH solution of twenty-four different starches. Nyman's [5] work also supports this view, he finding that the temperature at which the

[1] Lippmann, *J. prakt. Chem.*, **83,** 51 (1861) ; Lintner, *Wochensch. Brau.*, (1889), p. 285 ; Dox and Roark, *J. Am. Chem. Soc.*, **39,** 742 (1917) ; Francis and Smith, *Ind. Eng. Chem.*, **8,** 509 (1916) ; Stocks, *Brit. Assoc. Coll. Repts.*, **1,** 47 (1917) ; Harvey, *Am. J. Pharm.*, **96,** 752 (1924) ; Levites, *J. Russ. Phys.—Chem. Soc.*, **34,** 110 (1902) ; Herter, *Chimie et industrie*, **5,** 78 (1921) ; Kent-Jones, " Modern Cereal Chemistry " (Liverpool, 1927), p. 4 ; La Wall and Graves, *Trans. Wagner Free Inst. Sci.*, **7,** Part 2, 37 (1913) ; Baldwin, *J. Am. Chem. Soc.*, **52,** 2909 (1930).

[2] *Ind. Eng. Chem.*, **18,** 190 (1926).

[3] *7th Intern. Cong. Appld. Chem.*, London, 1909, **vi, a,** 7.

[4] *Schweiz. Apoth. Ztg.*, **61,** 654 (1923).

[5] *Z. Nahr. Genussm.*, **24,** 671 (1912).

granules swelled sufficiently to lose their bi-refrigence was higher the smaller the granules.

Alsberg could find no correlation between the original size of the starch granules and the final swollen granule. Potato starch swells about 40 times, canna starch about 16·5 times, both possessing rather large initial granules. Wheat starch and arrowroot are both small grained, but the former swells only about 8 times, whilst the latter attains about 45 times.

Alsberg and Rask [1] believe that gelatinisation of starch is a gradual change and not characterised by a definite temperature: "Gelatinisation is not a sharp transition point, but a long and gradual conversion process, which may or may not begin at the so-called gelatinisation temperature." They point out that: (*a*) intensive drying or wetting of starch; (*b*) the rate of heating the suspension, decidedly influence the gelatinisation temperature range; and also (*c*) the amount of water present must be adequate to permit complete gelatinisation.[2] The same authors experimentally observed that potato starch begins to swell at 50° C., further heating increasing the size of the granules up to a maximum just below 100° C. Their view is that "boiled starches are suspensions, not true colloidal solutions at all, unless they have been severally boiled or otherwise treated to disintegrate the suspended particles. Within the suspended swollen granules there is a colloid, probably colloidally dissolved." [3]

Taylor and Beckmann [4] produce experimental evidence, which supports Alsberg's views. They showed that grinding a warm starch paste renders it more fluid. Intensive dispersion of the paste by means of an homogeniser gave a fluid so that "under the microscope even on stained samples, no characteristic granules could be seen in this paste, but the striations so characteristic of the whole granule were very much in evidence." Homogenisation apparently distorts but does not rupture the majority of the already swollen granules. It was shown too, by Taylor and Beckmann [5] that pastes of high viscosity give a large amount of insoluble constituent (Meyer's α-amylose), very limpid pastes showing a large amount of soluble constituent (Meyer's β-amylose). Experiment showed that "in mixtures of α- and β-amyloses in the proportion existing in corn starch in pastes containing 1 per cent. total solids, the viscosity was practically that of amylose alone and did not

[1] *Cereal Chem.*, **1,** 107 (1924).

[2] Cf. Rodewald, *Z. physik Chem.*, **24,** 193 (1897), and Rodewald and Kattein, *loc. cit.*, **33,** 579 (1900), who found that the heat of swelling of starch in water varies as the original moisture content of the starch, the greatest evolution of heat occurring during the absorption of the first portions of water and falling rapidly to zero when the moisture content is approx. 30 per cent.

[3] *Ind. Eng. Chem.*, **18,** 191 (1926).

[4] *J. Am. Chem. Soc.*, **51,** 294 (1929).

[5] *Ind. Eng. Chem.*, **18,** 713 (1926); *J. Am. Chem. Soc.*, **51,** 294 (1929).

approach that of a heat-gelatinised whole starch of the same concentration."

The authors contend that it is the non-disintegrated granule which regulates the viscosity of an ordinary starch paste, a view already put forward by Alsberg. The idea involved is that the viscosity is that of a suspension rather than a colloidal solution as, for example, in the case of gelatin. On this view boiled starch paste only results when the ratio : starch/water, permits close contact of the swollen granules ; dispersion by grinding starch in cold water fails to yield a paste. Alsberg finds that amylose alone will not yield a paste, neither will amylopectin. Paste formation, then, is a function of the anatomical structure of the *swollen* granule.[1]

The gelatinisation temperature of starch is lowered by the presence of alkalies and certain salts. Thus, Levites[2] found maize starch to gelatinise at 66°—68° C. in water, at 46°—48° C. in 10 per cent. NH_4CNS or KCNS, or in 20 per cent. NaI or KI, and at 52°—54° C. in 20 per cent. NaBr, KBr or $NaNO_3$. Gelatinisation occurs at ordinary temperature in 15—20 per cent. NH_4CNS or KCNS, 40 per cent. NaI or KI, or 50—60 per cent. NaBr or KBr. Reychler[3] observed gelatinisation of starch at ordinary temperature in 55 per cent. chloral hydrate, 29 per cent. $AgNO_3$ and 30—35 per cent. NH_4NO_3.

STARCH HYDROSOL

Treating starch with water below the swelling temperature removes appreciable amounts of carbohydrates and the pH value of the water changes in an irregular manner. Although the starch granules decrease in diameter, their appearance under the polarising microscope does not change, indicating that it is not necessary to rupture the granule to remove soluble amylose.[4]

Starch sols are prepared by heating thin pastes above the gelatinising temperature of the starch in question. When prepared by heating at 120° C. in an autoclave, a very viscous paste ensues, of low electrical conductivity. The viscosity is raised by alkalies and lowered by acids. On ageing, the viscosity falls almost to the value for water itself. The alkali or acid sols show similar but less pronounced ageing effect, with an increase in electrical conductivity, whilst a portion of the starch is flocculated as flakes, leaving a clear sol of low viscosity. For example, Stocks[5] found that a freshly-prepared and rapidly-cooled potato starch sol (2 per cent.) gave the following relative viscosity values (water = 1) : immediate value =

[1] Cf. also Nowopokrowsky and Tschebotarewa, *Kolloid-Z.*, **52,** 302 (1930).
[2] *J. Russ. Phys.-Chem. Soc.*, **34,** 110 (1902).
[3] *Bull. soc. chim. Belg.*, **29,** 118 (1920).
[4] Hess and Smith, *Ber.*, **62 B,** 1619 (1929).
[5] *Brit. Assoc. Coll. Repts.*, **1,** 47 (1917).

106·7 ; value after 5, 40, and 1,080 minutes = 96·3, 19·3, and 1·3 respectively.

The effect of high temperatures in preparing starch sols has been studied by Samec and Haerdtl,[1] who found that the sols prepared by heating 2 per cent. starch pastes for one hour at 120° C. may be almost clear (potato starch), somewhat cloudy (horse-chestnut or maize starch), or quite opaque (rice or wheat starch). The viscosity of such sols varies considerably, being high in the case of potato starch and low in the case of wheat or rice starch. The percentage of dialysable material may be high as with horse-chestnut starch(11), meadow saffron, arrowroot and turmeric starches (7), or low as with potato starch (2).

The authors give the following table of the influence of the temperature of solution on the properties of starch sols. In each instance, the 2 per cent. starch paste was heated for one hour at the temperature in question ; viscosities were determined with 1 per cent. sols at 25° C. and recorded relative to water as unity.

TABLE 19. PROPERTIES OF STARCH SOLS PREPARED AT VARIOUS TEMPERATURES.

Starch.	Temperature.	Appearance of Sol.	Relative Viscosity.	Dialysable Portion.	Iodine Colour of Dialysate.
				per cent.	
Potato	120° C.	Almost clear.	5·05	2	*Nil.*
	138° C.	Somewhat cloudy.	2·51	2	*Nil.*
	155° C.	Clear ; yellow.	1·85	5·5	Dark blue.
Wheat	120° C.	Almost opalescent.	2·10	3	Blue-green.
	138° C.	Cloudy.	1·73	4	Blue.
	155° C.	Cloudy ; yellow.	1·45	8	Dark blue.
Maize	120° C.	Somewhat cloudy.	1·60	4	Violet.
	138° C.	Almost clear.	1·53	4	Blue.
	155° C.	Almost clear.	1·40	6	Dark blue.
Rice	120° C.	Somewhat cloudy.	1·48	5	Violet.
	138° C.	Cloudy.	1·48	5	Blue.
	155° C.	Cloudy deposit.	1·09	4·5	Blue-violet.

The effect of the time of heating 2 per cent. potato starch pastes at various temperatures on the physical properties of the sols so

[1] *Kolloidchem. Beihefte*, **12**, 283 (1920).

produced has been investigated by Samec and Jenčič,[1] who published the following table :—

TABLE 20. INFLUENCE OF HEATING STARCH SOLS.

Temperature.	Property	Duration of Heating in Hours.					
		1	2	3	4	6	8
120° C.	Relative viscosity . .	7·16	3·75	—	2·48	1·70	1·46
	Electrical conductivity × 10^5	4·70	5·76	—	6·19	6·37	6·80
	Percentage ultra-filterable .	7	5	—	7	10	10
135° C.	Relative viscosity . .	3·23	—	2·12	—	1·85	—
	Electrical conductivity × 10^5	5·56	—	6·59	—	6·90	—
	Percentage ultra-filterable .	7	—	7	—	8	—
150° C.	Relative viscosity . .	2·23	—	1·38	—	1·10	—
	Electrical conductivity × 10^5	6·51	—	8·58	—	11·78	—
	Percentage ultra-filterable .	10	—	16	—	60	—

Electrolytes do not influence starch sols until the effect of ageing becomes pronounced.[2] The hydrosols are negatively charged,[3] due to the amylopectin content. The electric charge decreases greatly in old or ash-free sols, due probably to a change in the character of the amylopectin.[4] Samec [5] has followed the ageing of starch sols by a study of the Brownian motion which gradually ceases, simple vibration attaining and finally all movement ceasing. The ultra-microns aggregate to larger particles, giving rise to either a spheroidal aggregation or a string-like aggregation, the former being exhibited in those starch sols giving a blue colour with iodine, the latter with sols giving a red colour.

Alkali peptises starch, a phenomenon investigated in detail by Samec [6] and others.[7] Phosphate appears to stabilise starch sols.

Ageing is much more rapid in hot solutions and the decrease in viscosity is accompanied by a corresponding decrease in (*a*) the amount of material removable by cataphoresis, (*b*) the amount of

[1] *Ibid.*, **7**, 151 (1915).
[2] But see Harrison, *J. Soc. Dyers Colourists*, **32**, 40 (1916).
[3] Clayton, *Kolloid-Z.*, **28**, 233 (1921).
[4] Samec and von Hoefft, *Kolloidchem. Beihefte*, **5**, 192 (1913).
[5] *Biochem. Z.*, **195**, 40 (1928).
[6] *Kolloidchem. Beihefte*, **8**, 33 (1916).
[7] Reychler, *Bull. soc. chim. Belg.*, **29**, 118, 309 (1920); Harrison, *loc. cit.*, also Ostwald and Frenkel, *Kolloid-Z.*, **43**, 296 (1927).

material precipitable by alcohol. Samec [1] points out the significant fact that " the three processes : ageing, heating under pressure, and demineralisation, produce one effect in common, *i.e.*, the transition of a gelatinising starch form into one incapable of gelatinisation, with the concomitant cleavage of phosphoric acid."

SOLUBLE STARCH

Numerous methods have been patented for the preparation of what is termed " soluble starch," *i.e.*, starch which readily dissolves in water. Heating starch under pressure with water, or heating with acids, alkalies, oxidising agents, or neutral salts provides a great variety of ways of accomplishing this change.[2] Soluble starch forms thin opalescent solutions in hot water. According to Samec and Jenčič [3] the size of the starch molecule is reduced (depolymerisation), and the molecular weight may be as low as 7740.[4] The subject has but little importance in food chemistry, where starch is usually regarded as a thickening agent.

SURFACE TENSION OF STARCH SOLS

No really comprehensive study has yet been made regarding the surface tension of starch solutions, but the available data indicate that starch *raises* the surface tension of water,[5] although there is no general acceptance of this.[6]

Harvey [5] gives the following surface tension data for starch solutions prepared by suspending the required amount of starch in distilled water, boiling for five minutes, making up the evaporation losses and cooling to the desired temperature in a covered vessel. The Du Noüy apparatus was employed.

SURFACE TENSION OF STARCH SOLS.
(dynes/cm.)

Temperature ° C.	Cassava Starch (1 per cent.)	Corn Starch (1 per cent.).	Potato Starch (1 per cent.).	Potato Starch (2 per cent.).
70	54·53	46·55	67·50	68·25
60	62·61	55·19	69·25	69·82
50	69·16	63·17	71·00	71·15
40	71·48	69·16	72·50	72·81
30	73·15	73·15	74·13	74·48
20	74·48	74·48	75·13	75·80

[1] Walton's " A Comprehensive Survey of Starch Chemistry " (New York, 1928), p. 54.

[2] See summary by Stocks, *loc. cit.*, p. 50 *seq.*, and by Samec. *loc. cit.*, p. 212 *seq.*

[3] *Kolloidchem. Beihefte*, **7**, 137 (1915).

[4] *Loc. cit.*, p. 441.

[5] Cf. Wo. Ostwald " Handbook of Colloid Chemistry " (London, 1919), p. 186; Harvey, *Am. J. Pharm.*, **96**, 816 (1924).

[6] Samec, *loc. cit.*, p. 296, believes starch solutions have surface tensions slightly *lower* than has water.

With regard to commercial soluble starch, Clark and Mann,[1] found the following values at 25° C.

Starch. Per cent.	Surface Tension. Dynes/cm.
Nil	72·05
0·01	72·36
0·1	72·26
0·5	72·13
1·0	71·98

THERMAL CONDUCTIVITY OF STARCH SOLS

Considerable importance is attached to the question of the thermal conductivity of starch sols and pastes, owing to its intimate

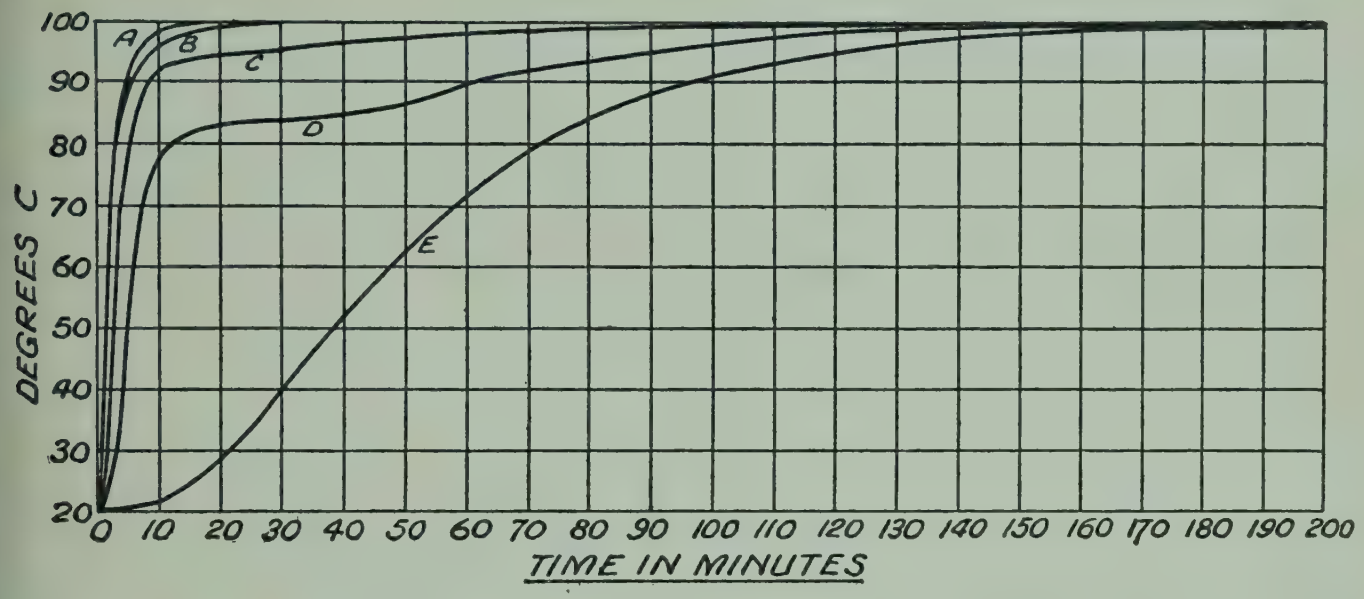


FIG. 14.—Time-temperature relations for 1 per cent., 2 per cent., 3 per cent., 4 per cent., and 5 per cent. starch solutions when processed in No. 3 tin cans at 100° C. Curves A to D were plotted from temperature readings made at intervals of 1 minute and curve E from readings at intervals of 5 minutes. Rise in temperature : A, for 1 per cent. solution ; B, for 2 per cent. solution ; C, for 3 per cent. solution ; D, for 4 per cent. solution ; E, for 5 per cent. solution.

relation to the heat penetration of starch-bearing foods during processing or sterilising operations.

Magoon and Culpepper [2] have published data for the rate of change of temperature of starch sols prepared by weighing out dried starch and adding sufficient water to prepare 1, 2, 3, 4 and 5 per cent. sols respectively. Such sols were heated on the steam bath for 1 hour with constant stirring, the evaporation losses made up, and the sols then cooled to 20° C. The sols were then filled into No. 3 cans (diameter = 4·25 inches, height = 4·87 inches, capacity = 35 fl. ounces) and processed at 100° C. Fig. 14, reproduced from the authors' bulletin, shows that the rate of change of temperature at the centre of the can is very little slower for 1 and 2 per cent. starch sols than for distilled water. In the case of a 3 per cent. sol, however, the initial rapid temperature rise suffers marked slowing down when the temperature at the centre of the can reaches 92° C.

[1] *J. Biol. Chem.*, **52,** 160 (1922).
[2] U. S. Dept. Agric., *Bull.*, **956,** 16 (1921).

with a 4 per cent. sol, the inflexion point is about 83° C., to be followed after 10—15 minutes by another, but slower, rise. The 5 per cent. sol shows a pronounced change in the type of curve, heat penetration being now very slow. The authors believe that in this case, conduction of heat replaces the convection of heat owing to the greater viscosity of the system.

Bigelow and his associates [1] have reported similar conclusions, concluding "that the conductivity of a solution of 6—10 per cent. of starch is almost as slow as the penetration of a saturated starch solution, and that heat penetration in the latter is due almost entirely to conductivity. The fluidity of these starch solutions is greatly increased by rising temperature. Notwithstanding this fact, convection currents do not appear to be effective in the more concentrated starch solutions."

Canned peas show a heat penetration almost as rapid as that of water. Should the sterilisation be intermittent, the heat penetration becomes slower with each successive period, inasmuch as starch comes into solution during heating.

VISCOSITY OF STARCH SOLS

The earlier work of Wo. Ostwald [2] showed that when a suspension of starch granules in water was heated, the viscosity rose to a maximum and then decreased, due to the greater dispersion of the system and to chemical changes. Boiling of starch sols results in a very rapid decrease in viscosity, very marked in the case of cassava starch and less so with corn starch.[3]

Yoshino [4] studied the viscosity of wheat starch in relation to concentration and temperature, using the Ostwald viscometer, and found Arrhenius' formula : $\log \eta/\eta_0 = \theta C$ to express the viscosity concentration results fairly accurately. Here $C = 100\ p/[100-p\ (n+1)]$, where p = gr. starch in 100 gr. sol, and n = the hydration factor. As the temperature is increased, the absorption of water decreases, and the following values of θ and n obtain :—

Temp.	θ	n
20° C.	0·412	12·6
30° C.	0·407	10·0
40° C.	0·407	7·6
50° C.	0·408	5·7

[1] Nat. Canners Assocn., *Res. Lab. Bull.*, **16—L,** 9 ; 29, 100 (1920).

[2] *Kolloid–Z.*, **12,** 213 (1913). See also the recent work of Chapman and Buchanan, *Iowa State Coll. J. Sci.*, **4,** 441 (1930) who also included studies on syneresis phenomena.

[3] Harvey, *Am. J. Pharm.*, **96,** 816 (1924).

[4] *J. Chem. Ind. Japan,* **26,** 870 (1923).

The following values of p, at 20°, 30°, 40° and 50° C. respectively, accord with infinite viscosity, *i.e.*, the sol becomes solid : 7·4, 9·1, and 11·6, and 14·9.

Rask and Alsberg [1] have published data relating to a comprehensive study of the viscosity of wheat starches, a subject much discussed in connection with the baking quality of wheat flours. They employed starches prepared from eleven flours from unblended wheats, and determined viscosity at 90° C. in concentrations of 2·85—5·75 per cent. (on a moisture-free basis). This temperature was chosen because : (1) starch pastes are no longer plastic, (2) this approximates to the baking temperature within a loaf of bread. In illustration of the type of results obtained, the following figures are quoted for two flours :—

Concentration of Starch (per cent.).		Viscosity in Centipoise.	
Sample A.	Sample B.	Sample A.	Sample B.
2·88	2·88	5·5	4·9
3·84	3·84	20·0	16·2
4·80	4·81	70·5	55·0
5·28	5·28	132·0	103·0
5·57	—	188·0	—

Plotting log. viscosity as ordinates, and the corresponding arithmetic concentrations as abscissæ, straight line graphs result, the corresponding type equation being $\log y = mx + \log b$. Here, y = viscosity, x = concentration of starch, m = slope, and b = intercept on the y axis.

The authors consider m and b to be characteristic of the particular starch which the equation represents, and m is probably a function of the colloidal properties of the starch such as dispersity, density, shape and water-imbibing properties of the starch granules. They were able to correlate the viscosity of the starch with the growing habit of the wheat, starches of the winter wheats showing the higher viscosities.

In a careful study of the viscosity of starches, Farrow and Lowe [2] observed that pastes prepared by boiling potato starch for two hours, on storage at 90° C. undergo a change which results in a decreased viscosity. Fig. 15, reproduced from their paper, illustrates the time-viscosity fall, with reference also to the concentration factor. The influence of the concentration on viscosity only

[1] *Cereal Chem.*, **1,** 7 (1924).
[2] *J. Textile Inst.*, **14,** 414 T (1923).

becomes pronounced at about 0·7 per cent. In the case of maize starch, storage produces but little change in viscosity, and the

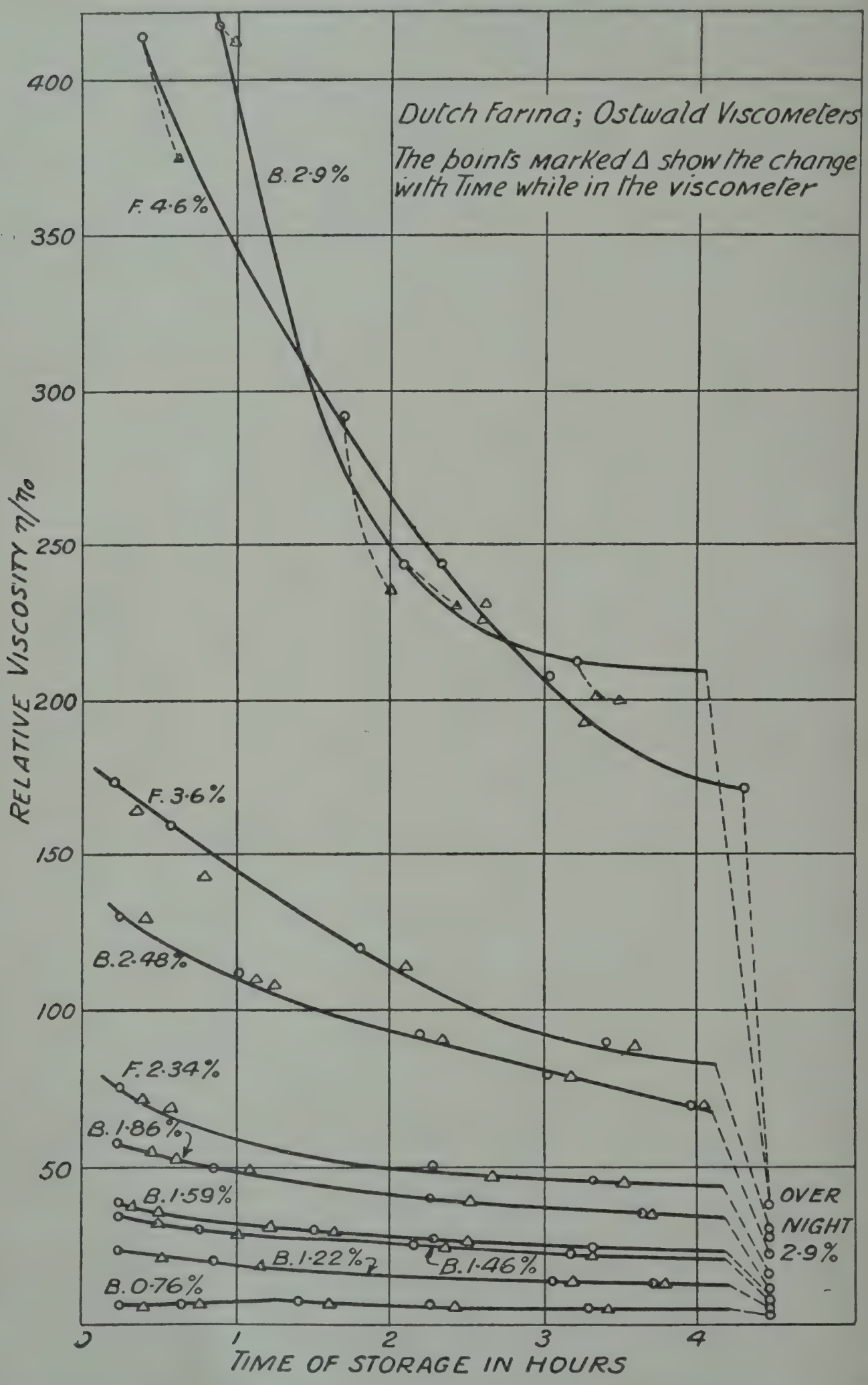


FIG. 15.—The time-viscosity fall of boiled potato starch stored at 90° C. The concentration factor is also illustrated.

concentration has to be about 1·5 per cent. before the viscosity of the paste reaches a value 4 times that of water.

Extensive investigations regarding the viscosity of starches in

general, and potato starch in particular, have been carried out in Samec's laboratory, especially with solutions prepared by heating starch and water under pressure. The following table by Samec and von Hoefft[1] shows the decrease in relative viscosity of potato starch by heating to 120° C., as a function of concentration and time.

Concentration of Sol.	Relative Viscosity after Heating.				
	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.
1 per cent. . .	6·08	2·11	1·40	1·28	1·27
2 ,, . .	5·91	3·22	1·70	1·39	1·27
4 ,, . .	5·40	2·84	1·87	1·44	1·27

The same authors[2] also give the following table, showing the decrease in viscosity of 1 per cent. sols at 25° C., consequent on heating 2 per cent. starch pastes at different temperatures for varying times before diluting for tests.

TABLE 21. RELATIVE VISCOSITY OF POTATO STARCH AS A FUNCTION OF TEMPERATURE AND TIME.

Temperature.	Duration of Heating in Hours.					
	1	2	3	4	6	8
100° C. . .	61·0	22·36	18·22	16·15	10·26	6·22
120° C. .	7·16	3·75	3·01	2·48	1·70	1·46
135° C. . .	3·23	2·75	2·12	1·92	1·35	1·22
150° C. . .	2·23	1·65	1·38	1·18	1·10	1·08

AGEING OF POTATO STARCH

The ageing of potato starch has been studied by Samec and von Hoefft,[3] who prepared sols by heating a 4 per cent. starch paste for 2 hours at 120° C., and then made dilutions to 2 per cent. and 1 per cent. sols. The sols were held at 15° C., 35° C., and 58° C. respectively; all viscosity determinations were made in 1 per cent. sols, diluting when necessary. The following results were obtained :—

[1] *Kolloidchem. Beihefte*, **5**, 171 (1913).
[2] *Ibid.*, p. 169.
[3] *Ibid.*, p. 176.

Table 22. Ageing of Potato Starch.

Concentration of Ageing Sol. (per cent.).	Age in Days.	Temperature at which Sol is Aged.					
		15° C.		35° C.		58° C.	
		Appearance.	Relative Viscosity.	Appearance.	Relative Viscosity.	Appearance.	Relative Viscosity.
1	0	Opalescent.	3·15	Opalescent.	3·15	Opalescent.	3·15
	1	Opalescent.	3·10	Turbid.	3·09	Very turbid.	3·03
	4	Opalescent.	2·95	Turbid.	2·95	Very turbid.	2·53
	11	Marked Opalescence.	2·72	More turbid.	2·79	Strongly turbid.	1·89
	20	Almost clear granular ppt.	2·46	More turbid.	2·65	Strongly turbid.	1·55
	31	Almost clear, granular ppt.	2·14	Less turbid.	2·54	Strongly turbid.	1·45
2	0	Opalescent.	3·15	Opalescent.	3·15	Opalescent.	3·15
	1	Opalescent.	3·16	Turbid.	3·06	Very turbid.	2·93
	4	Opalescent to turbid.	3·08	Very turbid.	2·97	—	—
	11	Opalescent to turbid.	2·88	Marked turbidity.	2·96	—	—
	20	Opalescent to turbid.	2·72	Clearer; precipitate commencing.	2·95	—	—
	31	Opalescent to turbid.	2·53		2·95	—	—
4	0	Opalescent.	3·15	Opalescent.	3·15	Opalescent.	3·15
	1	Very turbid.	1·92	Clear.	3·12	Turbid.	3·17
	4	Slimy mixture	—	Almost clear.	2·95	Turbid.	—
	11	Slimy precipitate.	—	Opalescent.	2·34	Turbid with granular precipitate.	2·58
	20	Slimy precipitate.	—	Slight opalescence.	1·83		2·44
	31	Very turbid.	2·26		1·71		—

INFLUENCE OF ACID AND ALKALI ON VISCOSITY

Whilst acids decrease the viscosity of starch sols on heating, alkalies increase it, as the following data by Samec and von Hoefft [1] clearly show. The results refer to potato starch pastes of 2 per

[1] *Ibid.*, p. 145. See also Freundlich and Nitze, *Kolloid-Z.*, **41**, 206 (1927).

cent. concentration heated to 120° C. The relative viscosities were determined at 25° C. on sols diluted to 1 per cent.

TABLE 23. RELATIVE VISCOSITY OF POTATO STARCH. EFFECT OF ACID AND ALKALI.

Hours Heated.	Neutral Sol.	$1N \times 10^{-4}$		$5N \times 10^{-4}$		$1N \times 10^{-3}$		$5N \times 10^{-3}$	
		HCl	NaOH	HCl	NaOH	HCl	NaOH	HCl	NaOH
1	6·85	6·70	7·11	6·35	7·84	5·80	7·31	4·55	9·45
3	3·85	3·75	3·95	3·50	4·12	3·41	4·16	3·38	6·41
6	3·35	3·34	3·35	3·33	3·42	3·32	3·54	3·32	5·93

The decrease in viscosity of starch under the combined influences of acid, processing and time of storage becomes a distinct problem in piccalilli, sauces and ketchups wherein starch is employed as a thickening agent. As the low viscosity arises from the breakdown of the starch it is of interest briefly to refer to an important paper by Fargher and Probert [1] on : " An Examination of the Early Stages of the Hydrolysis of Starch by Dilute Acids."

The temperature range examined was below 100° C. and above 40° C. Below 40° C. the hydrolysis is very slow. HCl and H_2SO_4 were chosen as acids, at temperatures of 40°, 50° and 60° C. It was found that the rate of hydrolysis of starch varied with the concentration of acid employed. The temperature effect was very marked, the rate of attack of starch being increased approximately four-fold for a rise in temperature of 10° C., *e.g.*, hydrolysis at 60° C. is 16 times as rapid as at 40° C.

As regards the nature of the starch the authors observe that " comparative experiments with potato, maize, wheat and rice starches show that they differ significantly, though not greatly, in their behaviour towards 0·2 N. sulphuric acid at 50° C., the differences gradually disappearing as the temperature is raised, until at 80° C. the rates of hydrolysis of rice and potato starches, which show the maximum difference at 50° C., become practically identical."

The hydrolysis was followed cuprimetrically. " In general, it has been found that for any given conditions of treatment there is an initial stage during which the starch is undergoing conversion into " soluble " starch. During this period the rate of formation of reducing substances gradually increases. Thereafter, it proceeds at a constant rate dependent upon the temperature and concentration of the acid." The formula, $N_{Cu} = k\,(T - t)$ expresses the later stage of the hydrolysis for a given concentration of acid. Here

[1] *J. Textile Inst.*, **18**, 559 T (1927).

N_{Cu} is the copper number of the hydrolytic products, T the time of reaction, and t the lag-period or time necessary to effect complete conversion of the starch into its soluble modification. Any pre-treatment of the starch bringing about its liquefaction will eliminate the lag-period.

The following table by the authors illustrates the course of hydrolysis for four different starches when heated at 50° C. with 0·2 N. H_2SO_4, the systems containing 1 per cent. of starch exactly. (For full experimental details the original paper should be consulted.)

TABLE 24. COMPARATIVE HYDROLYSES OF DIFFERENT STARCHES WITH 0·2 N. H_2SO_4 AT 50° C.

Time.	Copper Number.			
(Days).	Potato.	Maize.	Wheat.	Rice.
3	11·06	10·02	—	9·26
4	15·68	—	14·73	13·44
5	19·06	18·00	—	17·45
6	24·19	—	21·25	21·41
7	—	27·99	27·86	—
8	33·34	31·57	29·95	—
9	38·11	—	35·53	—
10	41·96	—	41·63	36·21
11	47·77	43·92	—	41·25
12	—	—	48·65	43·04
13	—	50·35	—	47·60
k	4·50	4·09	4·50	3·92

STARCH IODIDE

Reference has already been made to the iodine-colour which results when starch and starch products are treated with aqueous iodine. A voluminous literature [1] has grown dealing with this subject, but only the more modern aspects can here be considered.

[1] For summary to 1927 see Samec, " Kolloidchemie der Stärke " (Dresden and Leipzig, 1927), p. 314 *seq.*

The subject is of interest inasmuch as certain attempts have been made to utilise the colour reaction in analytical determinations of starch and because the phenomenon is so typically an illustration of adsorption by a colloid.

In the absence of water, iodine vapour colours rye, wheat and oat starches sepia, potato starch chocolate, and corn and rice starches violet.[1] In all cases the subsequent addition of water produces a blue colour. Again, starch treated with an alcoholic solution of iodine exhibits no colour change.

Barger [2] has shown that many organic compounds adsorb iodine, giving a blue colour. In the case of starch, the colour change depends on the degree of dispersion of the starch, relatively coarse particles giving a blue colour, and highly dispersed particles a reddish-brown colour.[3]

Mylius [4] early recognised that for an effective colour change, iodide must be present, but the work of Barger's laboratory showed that certain other salts are equally effective. Staiger,[5] emphasising that HI is necessary, points out that H_2S, Cl, $KMnO_4$ and tannic acid, all of which tend to remove iodine, prevent the colour phenomenon. There is evidence available that KI in the system forms KI_3, which is very easily adsorbed by starch.[6] In addition, the KI increases the dispersion of the starch.

That the adsorption isotherm applies to the "starch iodide" colour reaction is well recognised,[7] and a comprehensive investigation by Lottermoser and Ott [8] has quite recently confirmed this view, as also have the data of Wu and Wu.[9]

Baldwin [10] has made an interesting study of the phenomenon. He finds that gelatinised potato starch treated with iodine gives violet-stained grains, rapidly deepening and changing to blue. He was able to demonstrate that the violet sacs contained blue liquid. The temperature of gelatinisation of the starch influences the iodine colour of the grains. At 63° C. the material extracted from the grains was entirely blue, at 75° C. there was a trace of violet, but at 100° C. the violet predominated. It was further observed that the intensity of colour is a function of the amount of iodine (in KI solution).[11] Small amounts of iodine give a light blue colour, more

[1] Marino, *Ann. igiene.*, **35,** 8 (1926).
[2] *J. Chem. Soc.*, **89,** 1210 (1906); **101,** 1394 (1912); **107,** 411 (1915).
[3] Herzfeld and Klinger, *Biochem. Z.*, **107,** 268 (1920).
[4] *Ber.*, **20,** 689 (1887).
[5] *Z. Spiritusind.*, **50,** 300 (1927).
[6] Angelescu and Mirescu, *J. chim. phys.*, **25,** 327 (1928).
[7] Küster, *Ann.*, **283,** 360 (1894); Katayama, *Z. anorg. allgem. Chem.*, **56,** 209 (1908); Harrison, *Kolloid-Z.*, **9,** 5 (1911).
[8] *Kolloid-Z.*, **52,** 138 (1930).
[9] Alexander, "Colloid Chemistry" (New York, 1926), Vol. 1, p. 380.
[10] *J Am. Chem. Soc.*, **52,** 2911 (1930). Cf. Nowopokrowsky and Tschebotarewa, *Kolloid-Z.*, **52,** 303 (1930).
[11] Cf. Turner, *J. Am. Chem. Soc.*, **52,** 2768 (1930); Nichols, *Ind. Eng. Chem., Anal. Edn.*, **1,** 215 (1929).

iodine produces first violet and later red-violet. The spectroscope examination of the absorption bands proved that the original blue colour disappears with further addition of iodine and is replaced (not masked) by the deeper red-violet.

Fractionation of potato starch into amylose and amylopectin and treating the fractions with iodine led Sherman and Baker [1] to conclude that the iodine colour of amylose is 13–18 times more pronounced than with amylopectin. Baldwin observed that amylose + iodine yielded a blue colour, free from violet, whilst amylopectin + iodine yielded a red-violet colour, free from blue.

FLOUR

The United States Department of Agriculture defines [2] flour as follows :—

(a) Whole wheat flour, entire wheat flour, Graham flour, is the clean, sound product made by grinding wheat, and contains, in their natural proportions, all of the constituents of the cleaned grain.

(b) Flour, wheat flour, white flour, is the clean, sound, fine-ground product, obtained in the commercial milling of wheat, and consists essentially of the starch and gluten of the endosperm. It contains not more than 15 per cent. of moisture, not less than 1 per cent. of nitrogen, not more than 1 per cent. of ash, and not more than 1 per cent. of fibre.

Wheat flour has a variable composition depending on the grade of flour and on the blends of wheat employed. Kent-Jones [3] gives the following as the approximate general analysis of flour :—

	Per cent.
Starch	65—70
Proteins	9—14
Water	13—15
Cellulose and fat	1
Sugars	2·5
Ash	0·5

The detailed study of the various analyses of wheats and flours is admirably treated by Bailey [4] in his book, "The Chemistry of Wheat Flour." The present volume, however, is concerned solely with the colloid chemistry of the subject, particularly in relation to flour strength, staling of bread and other problems intimately connected with the colloid constituents of flour.

[1] *J. Am. Chem. Soc.*, **38,** 1885 (1916).

[2] Service and Regulatory Announcements, Food and Drug No. 2 (First Revision), Supplement No. 2, August, 1930.

[3] "Modern Cereal Chemistry" (Liverpool, 1927), p. 1.

[4] See also Hamill, Reports Local Govt. Board on Public Health and Medical Subjects (New Series **55**); Food Reports No. **14** (1911); Jago, "Technology of Bread-making" (Liverpool, 1921), Chap. 14.

When flour is kneaded with water and leavening agents it forms dough, the baking of which yields bread. No fixed analysis of bread can be given, but the general analytical range will be seen in the following table, which collects analyses of white breads.

TABLE 25. ANALYSES OF WHITE BREADS.

Water.	Protein.	Fat.	NaCl.	Other Ash.	Fibre.	Maltose.	Starch.	Analyst.
41·9	6·7	0·2	0·9	0·8	0·2	3·5	45·8	Plimmer.[1]
45·1	6·3	0·1	0·7	0·6	0·1	2·9	44·2	Plimmer.[1]
42·9	7·0	0·7	(1·1)		—	(48·3)		Cribb and Elliott.[2]
35·3	9·2	1·3	(1·1)		—	(53·1)		Sherman.[3]
35·0	8·0	0·75	1·0	0·5	0·30	(54·45)		Wiley.[4]
27·8	10·0	0·82	(0·85)		0·37	(60·70)		Novelli.[5]
Min. 26·39 Max. 47·90	4·81 8·69	0·1 1·0	(0·84) (1·4)		0·33 0·90	(38·93) (62·98)		Jago,[6] Pope,[7] Cox.[8]

The two main factors in flour are starch and protein, and as water-imbibing colloids they will affect the " viscosity " of dough and act as buffers in relation to the acid production during fermentation of dough.

pH VALUE OF FLOURS

The pH value of a flour extract is a little greater as a rule than pH 6, due mainly to the soluble phosphates and protein substances.[9] It has been common practice to determine the pH value on the filtrate or decanted liquid from a 1 in 10 flour-water suspension. Halton and Fisher,[10] however, recommend that the determination be made on the actual suspension,[11] this having a higher pH, from about 0·01 to 0·10 pH. No satisfactory reason is yet forthcoming for this fact. They observed that the pH value in flour extracts decreases as the ratio of flour to water increases, *e.g.*, the difference in pH between ratios of 1 : 10 and 1 : 2 for six Hungarian flours ranged from 0·08 (pH 5·94—5·86) to 0·25 (pH 6·29—6·04). The duration of extraction plays a small *rôle*, the pH decreasing as the

[1] " Analyses and Energy Values of Foods " (London, 1921), p. 191.
[2] *Ibid.*, p. 193.
[3] " Food Products " (New York, 1924), p. 326.
[4] " Foods and Their Adulteration " (London, 1917), p. 255.
[5] *Giorn. risiculture*, **5**, 68 (1915).
[6] *Loc. cit.*, p. 560.
[7] Allen's " Commercial Organic Analysis," Vol. I., (London, 1924), p. 586.
[8] " The Chemical Analysis of Foods " (London, 1926), p. 74.
[9] See the detailed paper by Bailey and Peterson, *Ind. Eng. Chem.*, **13**, 916 (1921).
[10] *Cereal Chem.*, **5**, 453 (1928).
[11] Cf. Whittier and Greve, *ibid.*, **6**, 161 (1929).

time of extraction is increased, *e.g.*, pH 6·02 after half-hour extraction and pH 5·88 after $4\frac{1}{2}$ hours extraction.

BUFFER VALUE OF FLOURS

The fermentation of flour in dough produces acid and the pH change will obviously vary with the degree of buffering of the flour constituents. The buffer action has usually been measured by ascertaining the pH change when a certain amount of acid is added to filtered or centrifuged flour extract. Since this only measures buffer action due to solutes, Halton and Fisher work with the actual flour-water mixture, finding that the apparent buffer action of a flour varies from 1·5 to 3·5 times that of the extract. Their method is as follows : " Into each of 200 c.c. stoppered bottles a 10 gr. sample of the flour is placed. To the first, 100 c.c. of distilled water is added ; and to the second, 90 c.c. of distilled water and 10 c.c. of N/100 lactic acid. After shaking at intervals for 30 minutes they are allowed to settle for at least 15 minutes." When sedimentation has been clearly defined, the pH value is determined on each, the difference between the two pH values being taken as the buffer value of the flour. Bailey [1] finds that the buffer value correlates with the ash content of flours.

VISCOSITY OF FLOUR SUSPENSIONS

In an endeavour to characterise flours in respect to their value for baking, various methods have been proposed to determine physico-chemical factors quantitatively and to correlate these with desirable or undesirable features of the flour. Viscosity [2] measurements have been prominent in this connection, and Gortner and Sharp [3] put forward the view that the viscosity of a flour-water suspension is a good index of the gluten therein to take up water, since " strong " gluten was believed to imbibe water, or become more heavily hydrated, than " weak " gluten. Probably glutenin is the responsible protein in the gluten. Other workers [4] have agreed that such viscosity measurements furnish a good index to loaf volume.

The viscosity of colloid systems in general is influenced by several factors, as discussed by Wo. Ostwald,[5] such as : (a) concentration, (b) temperature, (c) degree of dispersion, (d) solvation, (e) electric charge on the particles, (f) presence of electrolytes and

[1] " The Chemistry of Wheat Flour " (New York, 1925), p. 167.

[2] For the general principles involved see Lüers and Ostwald, *Kolloid-Z.*, **25,** 82 ; 116 (1919) ; Dunn, *Cereal Chem.*, **3,** 351 (1926) ; St. John, *ibid.*, **6,** 400 (1929) ; van der Lee, *ibid.*, **5,** 10 (1928) ; Denham *et al.*, *ibid.*, **4,** 206 (1927).

[3] *J. Phys. Chem.*, **27,** 481 ; 567 (1923) ; Minn. Agr. Expt. Sta., *Tech. Bull.*, **19,** 1 (1924).

[4] Heiduschka and Fichte, *Kolloid-Z.*, **32,** 193 (1923) ; Blish and Sandstedt, *Cereal Chem.*, **2,** 199 (1925) ; Lüers and Schwarz, *Z. Nahr. Genussm.*, **49,** 75 (1925) ; Morgan, *Cereal Chem.*, **1,** 288 (1924) ; Blair *et al.*, *ibid.*, **4,** 63, 206 (1927).

[5] *Trans. Faraday Soc.*, **9,** 34 (1913).

non-electrolytes, (g) thermal history and mechanical treatment of the system, (h) ageing of the system, (i) presence of some more viscous liquid. All these factors play a part in flour-in-water suspensions, with enzymic effects in addition.

In order to remove disturbing electrolyte effects, it is usual to determine the viscosity of flour-in-water systems, not on the suspension itself, but on the residual suspension after appropriate extraction with distilled water. Johnson [1] has investigated various factors involved in such extraction. Thus, the temperature of the water used in extracting influences the viscosity of the subsequent residue suspension, *e.g.*, " for each degree of difference in temperature there is a difference in viscosity of 7° MacMichael." The higher the temperature, the more electrolyte and soluble protein there are removed and the greater the increase in the hydration of the flour.

TABLE 26. VISCOSITIES OF FLOUR-IN-WATER SUSPENSIONS.

Temperature °C.	Viscosity Readings in ° MacMichael Viscometer.							
	Concentration of Flour-in-Water Suspensions in Gr. per 100 c.c.							
	14		16		18		20	
	A	B	A	B	A	B	A	B
15	39	10	61	17	97	25	119	36
20	45	11·5	73	18	104	28	139	39
25	54	13	80	21	116	31	162	48
30	69	16	106	24	154	35	210	48
35	76	18	115	25	167	33	230	44
40	82	20	122	26	173	34	255	45
50	89	21	149	26	226	34·5	326	46
55	140	33	265	49	410	73	585	107
60	380	48	625	70	1,005	103	1,810	176
70	265	—	465	—	—	—	—	—
80	86	—	154	—	—	—	—	—
96	—	—	196	—	—	—	—	—

A = Extracted at different temperatures for 1 hour with 1,000 c.c. of water and for 15 minutes with 500 c.c. of water.
B = Non-extracted suspensions maintained at different temperatures for 1 hour.

[1] *Cereal Chem.*, **4**, 87 (1927).

Increasing the time of extraction at lower temperatures will lead to the same results. Johnson noted that the degree of hydration undergoes a sudden change at 50° C. due to the increased swelling of gluten and the consequent decrease of free water. Again, he found that a non-extracted flour-in-water suspension has a lower viscosity than the corresponding extracted sample, pointing clearly to the influence of electrolytes (cf. ash). The table on p. 141 is based on Johnson's data and illustrates some of his findings concerning extraction.

Johnson and Herrington [1] have observed that the presence of CO_2 in the water used for extraction leads to an increase in the viscosities of acidulated flour-water suspensions. It is common practice to use acidulated suspensions for the viscosity determinations, the aim being to bring the system to that pH reaction where the viscosity will be a maximum. The actual reaction of the water used in carrying out the original extraction of electrolytes plays a pronounced part in determining the magnitude of the viscosity of the subsequent suspension of the flour residue. The following table from the authors' data illustrates this point and they mention that "approximately 50 per cent. of the nitrogen in the original suspension was decanted from the suspension which exhibited the highest viscosity on acidulation."

TABLE 27. VISCOSITIES OF ACIDULATED BLEACHED PATENT FLOUR SUSPENSIONS EXTRACTED AT DIFFERENT pH VALUES AND AT TWO DIFFERENT TEMPERATURES.

C.c. *N*/14 Acid or Alkali added.	25° C.			40° C.		
	pH	Viscosity.	Total N Extracted %	pH	Viscosity.	Total N Extracted %
Alkali 9	7·56	111	53·5	7·52	181	44·2
8	7·30	109	40·5	7·30	143	40·5
7	7·08	103	35·4	7·05	151	36·1
6	6·86	100	34·5	6·83	165	33·0
5	6·71	115	30·4	6·64	154	31·5
4	6·51	139	28·0	6·47	165	29·5
3	6·30	152	28·5	6·36	180	28·1
2	6·10	164	29·2	6·07	196	33·5
1	5·80	185	33·1	5·81	193	35·7
0	5·53	197	38·4	5·51	218	42·8
Acid 1	5·32	213	44·3	5·32	261	49·3
2	5·09	246	46·5	5·12	241	56·1
3	4·88	246	50·8	4·97	218	61·5
4	4·73	240	55·5	4·78	172	64·7
5	4·58	200	60·2	4·60	118	68·5
6	4·34	156	65·0	4·41	—	67·0

[1] *Ibid.*, **5,** 116 (1928).

The method employed was to place 18 gr. flour in 1,000 c.c. distilled water and add portions of N/14 HCl or NaOH. After settling the clear liquid was decanted and another lot of 500 c.c. distilled water added, to be decanted 15 minutes later. The residue was now made up to 100 c.c., 0·5 c.c. of 20 per cent. lactic acid added, and the viscosity determined with the MacMichael viscometer.

VISCOSITY AND PROTEIN QUALITY

Gortner[1] has pointed out that the viscosity of flour-in-water suspensions is the resultant of several factors, the most important being the quantity and quality of the proteins present. The quantity and quality factors both vary in different flours, and hence it does not necessarily follow that absolute viscosities are related to the quality of the gluten and a correction is necessary in order to gain the true relation. His method is to determine the viscosities of flour-in-water suspensions as a function of the concentration of flour and plot as abscissæ the log. of viscosities and as ordinates the log. of flour concentration. The graphs are then straight lines conforming to the general equation :—

$$\text{log. viscosity} = a + b\,(\text{log. flour concentration}).$$

Of the constants, a and b, the first determines the position of the graph on the viscosity axis at zero flour concentration, whilst b is the tangent of the angle made by the graph with the abscissa. Thus, " b is directly related to the slope of the line and may be taken as a numerical value of gluten quality."

The values of the constants may be calculated by the method of least squares, using the formulæ :—

$$a = \frac{\Sigma(x).\Sigma(xy) - \Sigma(x^2).\Sigma(y)}{[\Sigma(x)]^2 - n\Sigma(x^2)}$$

$$b = \frac{\Sigma(x).\Sigma(y) - n\Sigma(xy)}{[\Sigma(x)]^2 - n\Sigma(x^2)}$$

Where Σ = sum of values.
x = log. flour concentration used.
y = log. viscosity found.
n = number of observations, *i.e.* with different flour concentration.

For the experimental technique the original paper should be consulted, but in brief it is as follows : suspensions of flour are made with 12, 15, 18 and 21 gr. in 100 c.c. distilled water and when smooth 900 c.c. distilled water added. Shake at intervals for 45 minutes, settle for 15 minutes and decant. To the residue add 500 c.c. distilled water, shake and then settle for 15 minutes. After

[1] *Ibid.*, **1,** 75 (1924) ; *vide* Blair *et al.*, *ibid.*, **4,** 63 (1927).

decantation, the residue is washed into a 100 c.c. cylinder and the volume made up to 100 c.c. with distilled water. This well-mixed suspension is placed in the viscometer cup and 0·5 c.c. 20 per cent. lactic acid added, thus adjusting the system to approximately pH 3, where viscosity is a maximum. The viscosity is then determined, usually with the MacMichael torsion instrument.

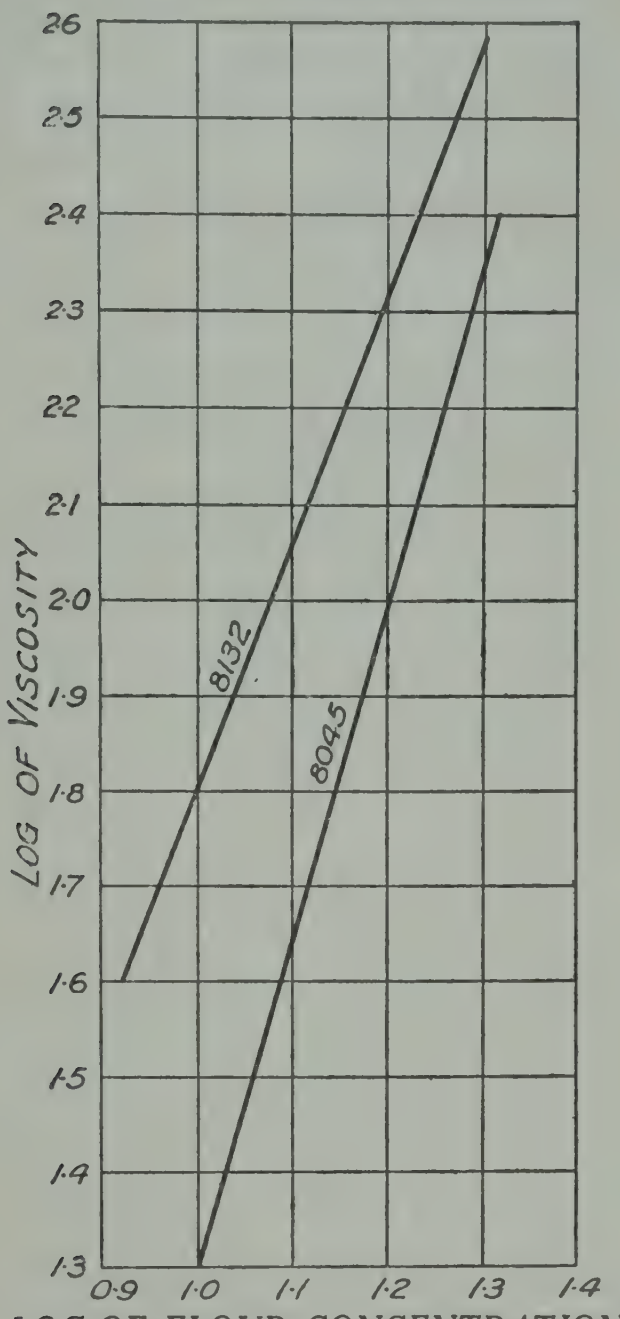


FIG. 16.—Logarithmic viscosity-concentration curves of two flours differing in gluten quality.

Fig. 16, reproduced from Gortner's paper gives the graphs corresponding to the data in the table on p. 145.

The protein quantity of the two flours obscures the quality factor so far as absolute viscosities are concerned, but the quality constant, b, is unaffected. The loaf volumes differ but little in spite of the wide variation in protein quality so that "the much smaller quantity of high quality protein was approximately equal, so far as loaf volume is a measure, to a much larger quantity of medium quality protein."

Numerous determinations of b have been published by Bailey and Johnson,[1] which reflect the marked influence of the non-removal of electrolytes from flour-in-water suspensions. Thus, taking from their fourteen flour samples two, one with low and one with high protein content, the following figures illustrate the matter :—

Flour.	Ash.	Protein.	Quality Constant (b).	
Sample.	Per cent.	Per cent.	Electrolytes Extracted.	Electrolytes not Extracted.
A	0·57	7·92	3·15	3·21
B	1·71	13·11	2·57	3·69

Blish and Sandstedt [2] also determined b as a measure of gluten quality for nineteen flours and failed to find any useful correlation

[1] *Ibid.*, **1**, 340 (1924).
[2] *Ibid.*, **2**, 191 (1925).

TABLE 28. VISCOSITIES OF FLOURS IN RELATION TO PROTEIN QUALITY.

Flour.	Weight (Dry Basis).	Protein (Dry Basis).	Angle of Graph with Abscissa.	Loaf Volume.	Viscosity ° M.	Log. Concn. (x).	Log. Viscosity (y).	b
A	10·546	9·75 %	73° 47′	1840 c.c.	23	1·0233	1·3617	3·44
	13·182				56	1·1199	1·7482	
(8045)	17·576				133	1·2450	2·1239	
	20·212				219	1·3056	2·3404	
B	8·767	14·27 %	68° 21′	1880 c.c.	42	0·9428	1·6232	2·52
	10·520				79	1·0220	1·8976	
(8132)	13·1510				138	1·1189	2·1399	
	15·781				203	1·1981	2·3075	
	18·411				281	1·2650	2·4487	

between b and loaf volume even among flours of similar protein content. It is their opinion that all the factors concerned in the viscosity of a flour-in-water suspension are not yet elucidated, and the method does not at present constitute a universal means of estimating flour strength. Similarly, Smith [1] found that although all his flours of high viscosity had excellent baking quality, flours of low viscosity showed variable baking qualities from poor to excellent. He concluded: "Viscosity is not a measure of baking quality, except possibly in the case of short and standard patents, within each of which grades there seems to be some correlation between viscosity and baking quality."

Kent-Jones,[2] whilst admitting the value of viscosity measurements, believes "the only danger lies in pushing the results too far." Obviously, considerable research is still required on the problem, and, for the present, loaf volume seems the safest guide to the baking quality of a flour from the commercial standpoint.

PROTEINS IN FLOUR

The pioneer work of Osborne [3] led to the conclusion that the wheat kernel contained five proteins differing in composition, solubility, etc. These simple proteins were: a prolamine (gliadin), a glutelin (glutenin), an albumin (leucosin), a globulin and a proteose. The leucosin occurs to the extent of 0·3–0·4 per cent. of the wheat kernel, the globulin to 0·6 per cent., whilst the proteose is present only in very small amounts. Numerous investigations have since followed and the general consensus of opinion is that although wheat flour contains gliadin, glutenin and leucosin, the proteose is absent and the presence of a globulin is questionable.[4]

GLIADIN

This protein, of molecular weight reported as 12,460 [5] and 20,700 [6] (minimal value), constitutes about 50 per cent. of the total protein of flours and its composition is constant according to the findings of various investigators.[7]

Its preparation [8] from wheat flour commences with the removal of starch from the gluten in dough and the subsequent extraction

[1] *Ibid.*, **2,** 190 (1925).
[2] "Modern Cereal Chemistry" (Liverpool, 1927), p. 145. See also Mohs, *Chem.-Ztg.*, **47,** 889 (1923).
[3] Carnegie Institution of Washington, *Pub.*, No. **84** (1907).
[4] Hoffman and Gortner, *Cereal Chem.*, **4,** 228 (1927).
[5] Ashmarin, *Russ. Arch. Biol. Sci.*, **23,** 327 (1924).
[6] Cohn *et al.*, *J. Biol. Chem.*, **63,** 721 (1925).
[7] But see Blish and Pinckney, *Cereal Chem.*, **1,** 315 (1924).
[8] For full practical details see Hoffman and Gortner, *loc. cit.*, p. 222; Blish and Sandstedt, *ibid.*, **3,** 144 (1926); Dill and Alsberg, *J. Biol. Chem.*, **63,** LXVII. (1925); Tague, *J. Am. Chem. Soc.*, **47,** 419 (1925).

of the gluten (in fragments) with 70 per cent. alcohol at ordinary temperature. After concentrating the clear alcoholic filtrate to a syrup, the gliadin is precipitated by pouring the syrup into water. The crude gliadin is redissolved in 70 per cent. alcohol, filtered and again precipitated. Further purification is effected by redissolving in 70 per cent. alcohol and then pouring the clear filtrate into a mixture of absolute alcohol and ether. Washing the precipitated gliadin with ether and then drying yields a pure white product.

When gliadin is suspended in distilled water [1] turbid or opalescent systems result, extremely sensitive to acid or alkali, the gluten swelling [2] and undergoing hydration and, in general, functioning amphoterically as other proteins. The isoelectric point is at pH 6·5.[3]

Tague [4] has investigated in considerable detail the question of the solubility of gliadin in aqueous solutions of acids, alkalies and salts. He found that acetic acid is the best solvent. When using HCl or H_2SO_4, maximum solubility occurs at about pH 2·0 and minimum solubility at about pH 6·5, with a second minimum at about pH 0·0. The solubility of gliadin was found to vary with the manner of its preparation, and Tague advises against the use of methyl alcohol, hot ethyl alcohol and intensive drying. NaOH solutions readily dissolve gliadin, whereas solutions of Na_2CO_3 are not good solvents. Aqueous solutions of neutral salts, with the exception of $MgCl_2$, are poor solvents. In aqueous methyl alcohol, the maximum solubility at 25° C. occurs in 70 per cent. (by volume) alcoholic system, the gliadin solution containing 132·2 mgms. gliadin per 100 c.c.

GLUTENIN

There is some controversy regarding the individuality of this protein in wheat flour, as its fractionation is claimed by Halton [5] and Csonka and Jones,[6] whilst other workers [7] do not agree that fractionation is substantiated.

Its preparation [8] follows the removal of gliadin from gluten, as outlined above, the residue being taken up in 0·5 per cent. NaOH, and the solution clarified by supercentrifuging (Hoffman and Gortner's method). Addition of acid to pH 4·5—5·0 causes precipitation of the protein, which can be purified by redissolving in 0·5 per cent. NaOH and again precipitating with acid. Final purification

[1] Lüers, *Kolloid-Z.*, **25,** 177 (1919).
[2] Cf. von Fellenberg, *Mitt. Lebensm. Hyg.*, **10,** 229 (1919).
[3] Tague, *J. Am. Chem. Soc.*, **47,** 418 (1925); *Cereal Chem.*, **2,** 202 (1925). Cf. Johnson and Herrington, *ibid.*, **5,** 43 (1928).
[4] *Cereal Chem.*, **2,** 117 (1925).
[5] *J. Agr. Sci.*, **14,** 587 (1924).
[6] *J. Biol. Chem.*, **73,** 321 (1927).
[7] Cf. Blish, *Cereal Chem.*, **2,** 127 (1925).
[8] For full details see Hoffman and Gortner, *Cereal Chem.*, **4,** 222 (1927); Blish, *ibid.*, **2,** 127 (1925); Blish and Sandstedt, *ibid.*, **2,** 55 (1925); Blish, Abbott and Platenius, *ibid.*, **4,** 129 (1927); Tague, *J. Am. Chem. Soc.*, **47,** 419 (1925); Larmour, *J. Agr. Research*, **35,** 1091 (1927).

is effected by solution in 0·5 per cent. NaOH, adding four volumes of methyl alcohol and precipitating from the filtrate by addition of dilute HCl. Any trace of gliadin is removable by extraction with 70 per cent. alcohol.

The minimal molecular weight of glutenin is given by Cohn [1] as 36,300, and the isoelectric point [2] as pH 6·8—7·0. Though practically insoluble in distilled water, glutenin is peptised by dilute acids and alkalies,[3] maximum peptisation [4] occurring at pH 3 and pH 11.

THE PEPTISATION OF WHEAT PROTEINS

Extensive investigations of much importance have been carried out by Gortner and his associates [5] (see p. 43) on the question of the peptisation of wheat flour proteins by solutions of inorganic salts. They chose twelve wheat flours of great diversity as regards type and geographical origin, and made extractions with a series of inorganic salts of different concentrations. The aim was to study the varying extent of protein extraction in relation to : (1) the nature of the salt solution used and the proteins of any one flour, (2) the nature of the salt solution used and the proteins of a series of flours. The following conclusions were reached :—

(a) Employing different salt solutions in equivalent ionic concentration, a given wheat flour responds differently as regards the amount of extractable protein.

(b) Confining extraction to one salt solution, but using different flours, there is found a wide variation in the amount of peptisable protein.

(c) Such variations are not a function of pH, but depend on : (1) the ease of peptisation of the proteins in a given flour, (2) definite anionic and cationic effects arising from the salts employed.

(d) The " salt-soluble protein " fraction of a flour has a varying composition depending on the salt employed.

(e) The above variations reflect those colloidal properties of wheat flour proteins which are functions of biological and environmental conditions.

Gortner [6] points out that " all of the various protein fractions which have been considered to be present in wheat flour and which are regarded as fixed entities, can be isolated in a constant proportion, providing that a prescribed routine procedure for protein isolation is followed." Deviation from such standard methods will give different results, since we are not dealing with solubility in the

[1] *J. Biol. Chem.*, **63,** 721 (1925), whilst Bailey, " The Chemistry of Wheat Flour," p. 252, gives 108,900 as the probable molecular weight.
[2] Tague, *J. Am. Chem. Soc.*, **47,** 418 (1925).
[3] Cf. Henderson and Cohn, *J. Am. Chem. Soc.*, **40,** 857, 867 (1918).
[4] Sharp and Gortner, *J. Phys. Chem.*, **27,** 942 (1923).
[5] *Cereal Chem.*, **6,** 1 (1929).
[6] " Outlines of Biochemistry " (New York, 1929), p. 365.

normal sense, but with the peptisation of colloid micelles behaving as typical lyophilic systems.

Recently, Blish,[1] in an essay entitled "The Gluten and Non-Gluten Proteins," has summarised the main literature to date. Gluten is usually considered as a mixture of the two proteins gliadin and glutenin. The relation of these mixed proteins to flour quality in baking is difficult to define, and there is a voluminous and not yet conclusive literature on the problem. His conclusion is that valid methods for the identification and quantitative separation of individual flour proteins, with the exception of gliadin, are lacking, and although strong tendencies toward certain *types* of chemical constitution and properties can be demonstrated, "beyond this the evidence is uncertain and indefinite."

How far this difficulty is met with in attempting a quantitative correlation of the protein content of flour with its baking quality or strength will be evident in the ensuing discussion.

THE STRENGTH OF FLOUR

The general idea regarding the strength of a flour is that a strong wheat gives a flour which, on baking results in large well-piled loaves, *i.e.* loaves of a smooth and finely vesiculated texture.[2] Martin,[3] Remington[4] and Kent-Jones[5] have drawn attention to the inadequate scope of this definition, and the latter worker[6] advances the more scientific definition that "strength may be regarded as the factor which will normally produce large well-piled loaves, provided that the gas production is sufficient, and that in practice this can often best be judged from the behaviour and the properties of the fermented dough. This means a sufficiency of protein,[7] the degree of dispersion of which lies between two useful colloidal limits."

It has long been recognised that the strength of flour was intimately associated with its gluten content,[8] but experience showed that the "quality" of the gluten was often more important than its quantity.[9] When Osborne[10] showed that gluten could be separated into two proteins, gliadin and glutenin, on the basis of their different

[1] *Cereal Chem.*, **7**, 421 (1930).
[2] Humphries and Biffen, *J. Agr. Sci.*, **1**, 1 (1906).
[3] *J. Soc. Chem. Ind.*, **39**, 246 T (1920).
[4] *Ind. Chem.*, **2**, 35 (1926).
[5] *Food Manfr.*, **1**, 101 (1927).
[6] "Modern Cereal Chemistry" (Liverpool, 1927), p. 133.
[7] *Vide* Bailey and Sherwood, *Cereal Chem.*, **3**, 393 (1926).
[8] *Vide* Hall, *J. Board Agr.*, **11**, 331 (1904); Balland, *Compt. rend.*, **123**, 136 (1896); Shutt, Central Exp. Farm (Canada), *Bull.*, **57** (1907), and *Proc. 7th Intern. Cong. Appld. Chem.*, Section VII., 108 (1910); Schneidewind, *Z. ges. Getreidew.*, **1**, 7 (1909).
[9] *Vide* Snyder, U.S. Dept., Agr., *Bull.*, **101** (1901).
[10] Osborne and Voorhees, *Am. Chem. J.*, **15**, 392 (1893); **16**, 524 (1894).

solubilities in dilute alcohol, the way was opened for a new method of attack on the problem of strength. Earlier workers [1] concluded that strong flours were characterised by a high gliadin-glutenin ratio, but it was later recognised that this contention was not always supported by baking practice.[2]

The work of Wood [3] was directed to (a) the size of the loaf (influenced by the gas given off during the fermentation of the dough), (b) the shape of the loaf (influenced by the electrolyte content of the flour, this affecting the condition of the gluten). He observed that the gluten was " dispersed " and rendered weak and inelastic by small amounts of acids and bases, salts having the opposite effect. He found, too, that the volume of the loaf was proportional to the rate of evolution of CO_2 in the later stages of dough fermentation, *i.e.* proportional to the amount of available sugar. Alway and Hartzell [4] could not confirm that loaf volume and gas volume were proportional, whilst Shutt [5] found no relation between loaf-volume and sugar content. Bailey [6] believes that " the strength of flour is determined by the ratio between the rate of production of CO_2 in, and the rate of loss of CO_2 from, the fermenting mass of dough."

Upson and Calvin [7] studied " The Colloidal Swelling of Gluten " and found that in solutions of lactic, acetic and hydrochloric acids, ranging from 0—0·5 N., there was initially a rapid absorption of water, reaching a maximum at 0·01 N., 0·04 N., and 0·005 N. acid respectively, and then a fall off as the acid concentration rose, this being most marked with HCl. In 0·2 N. and 0·5 N. HCl, the moist gluten *gives up* water. Addition of salts such as K. tartrate, K_2HPO_4, KCl, $CaCl_2$ and $NH_2.CH_2.COOH$ to the lactic acid solutions greatly depressed the swelling, this effect paralleling the increasing concentration of the salt. The water absorption is reversible and is increased by rise in temperature. These authors concluded [8] that quality [9] of gluten was important as well as quantity in bread-making, the quality of gluten being regulated by the nature and concentration of the acids and salts present in the dough. The electrolytes should be such as to inhibit water absorption by the gluten.

[1] Fleurent, *Compt. rend.*, **123,** 755 (1896); Guess, *J. Am. Chem. Soc.*, **22,** 263 (1900); Snyder, Minn. Expt. Sta., *Bull.*, **62** (1899). See also Casaubon, *Bull. assocn. chim. sucr. dist.*, **47,** 44 (1930); Girard, *Compt. rend.*, **123,** 327 (1896); Kosutany, *Z. ges. Getreidew.*, **2,** 36 (1910); Kuhn and Richter, *Kolloidchem. Beihefte,* **22,** 421 (1926); Norton, *J. Am. Chem. Soc.*, **28,** 8 (1906).

[2] Konig and Rinteln, *Z. Untersuch. Lebensm.*, **8,** 721 (1904); Wood, *J. Agr. Sci.*, **2,** 139, 267 (1907); Rammstedt, *Z. ges. Getreidew.*, **1,** 285 (1909).

[3] *J. Agr. Sci.*, **2,** 139, 293 (1907).

[4] Nebraska Agr. Expt. Sta., *Ann. Repts.*, 23 (1909).

[5] *Canadian Miller and Cerealist,* **5,** 176 (1913).

[6] *Ind. Eng. Chem.*, **8,** 53 (1916); **14,** 147 (1922).

[7] *J. Am. Chem. Soc.*, **37,** 1295 (1915).

[8] Nebraska Agr. Expt. Sta., *Res. Bull.*, **8,** 3 (1916).

[9] *Vide* comments on this investigation by Lüers and Ostwald, *Kolloid-Z.*, **27,** 34 (1920).

Olson,[1] however, could find no correlation between the gluten content and water-retaining properties of flour, but agreed that the volume of a loaf was inversely proportional to the gluten content. He also supported Woods' contention that the ratio of soluble ash to protein determines the shape of the loaf, shape being influenced by the nature of the electrolytes present.

Gortner and Doherty [2] extended the work of Upson and Calvin, examining the hydration capacity of gluten from strong and weak flours. In water alone, no appreciable difference in hydration was observed, but in acids the glutens from weak flours had a lower rate of hydration and a lower maximum. Glutens from weak flours disintegrated more rapidly than did the glutens from strong flours, when in water or in acid solutions. Further experiments by Sharp and Gortner [3] have substantiated these results. The interesting fact was elucidated that drying the glutens *in vacuo* at 45—50° C. caused a pronounced change in colloidal properties and made the glutens more akin. This accords with the view that strong gluten is a more perfect colloidal gel than weak gluten. Should the percentage of gluten in flour be reduced by adding starch, the gas-producing power is not necessarily impaired, but the gas-retaining power is weakened. Such impairment also follows treatment of the flour with 96 per cent. alcohol and with water, because the colloidal condition of gluten is altered.[4]

Martin [5] in a detailed study of the factors concerned in flour strength found that a strong flour must possess (*a*) a minimum gas-producing capacity, and (*b*) a high gas-retaining capacity. The first is controllable by adding an amylolytic enzyme, whilst the second is dependant on the amount and form of the proteins in the flour. It was observed that flours with high gas-retaining capacities and high bakers' marks were high in their content of "amended gliadin," *i.e.*, the crude gliadin less the water-soluble proteins after a 3 hours' extraction with water.

That the more gliadin a flour contains the better is its baking quality has been agreed by many workers. Von Fellenberg [6] found that a tough, gummy, elastic mass resulted when gliadin swelled in water, so that dough consists of a gummy network of swollen gliadin, dispersed in which are glutenin and starch granules. The retention of CO_2 during fermentation and baking is thus accounted for. Addition of dextrin or gum to flour results in a poor dough, probably because the gliadin-water equilibrium is adversely affected.

Lüers has made extensive investigations on gliadin and believes

[1] Wash. Agr. Expt. Sta., *Bull.*, **144,** 5 (1917).
[2] *J. Agr. Research,* **13,** 389 (1918).
[3] *J. Phys. Chem.*, **26,** 101 (1922).
[4] Johnson and Bailey, *Cereal Chem.*, **2,** 95 (1925).
[5] *J. Soc. Chem. Ind.*, **39,** 246 T (1920).
[6] *Mitt. Lebensm. Hyg.*, **10,** 229 (1919).

that this easily swollen protein acts as a protective colloid for glutenin, but its properties are greatly influenced by the climatic conditions obtaining during the ripening of the wheat.[1] Since some specimens of gliadin swell slowly and require more water than others, time and concentration become important factors in preparing dough. Since gliadin is acid-sensitive, the hydrogen ion concentration should be kept as near as possible to 10^{-5}. New flour is not acid enough, whilst old flour is frequently too acid because of enzyme action. NaCl in dough reduces gliadin swelling and therefore old flour stands more salt.

In a later paper [2] Lüers points out that acid or alkali raises the degree of hydration of gliadin, accompanied by a rise in viscosity. The hydration and viscosity subsequently fall if the acid content rises too high. The hydration is strongly inhibited by neutral salts. An optimum degree of hydration ensues, which is *not* the maximum hydration.

According to Woodman [3] the gliadins from strong and weak flours are identical proteins, but the glutenins therefrom are different. He believes loaf size varies as the diastatic capacity of the flour, whilst the shape of the loaf depends on the physical properties of the gluten, especially on the nature of the glutenin therein.

On the basis of the different specific rotations of the alcohol-insoluble fractions of wheat glutens, Halton [4] concluded that strength in flour is associated with a glutenin of high specific rotation, weakness with a glutenin of low specific rotation. His evidence, however, is somewhat unsatisfactory.[5]

Dearsley [6] made analyses and baking tests on five strains of New Zealand wheat and decided that the ideal flour is one possessing a high proportion of gluten and which, on being made into dough, has a pH value in the dough of approximately 5. Doughs of higher initial pH values ferment more slowly. The loss of water in the dough on baking and in the bread on ageing is greatest when the loaf is made from flour containing gluten of poor quality. Similarly, Foster [7] correlated the protein content of flour with the quality and strength of the flour, although in two instances the factor of quality of gluten gave superior flours even with low protein content. Again,[8] he found that the hydration capacity of the moist gluten did not always indicate the quality of the flour as judged by baking tests, but

[1] Cf. Swanson, *Cereal Chem.*, **1,** 279 (1924).
[2] *Chem.-Ztg.*, **44,** 324 (1920).
[3] *J. Agr. Sci.*, **12,** 231 (1922); see the later work of Blish and Pinckney, *Cereal Chem.*, **1,** 309 (1924).
[4] *J. Agr. Sci.*, **14,** 587 (1924).
[5] Blish, *Cereal Chem.*, **2,** 127 (1925).
[6] *N. Zealand J. Sci. Tech.*, **8,** 34 (1926).
[7] *N. Zealand J. Agr.*, **32,** 26 (1926).
[8] *Loc. cit.*, **32,** 93 (1926).

hydration capacity was closely related to the *texture* of the loaf.[1] Although there was no relation between the initial pH value of the flour and the loaf volume, it was found that in flours of approximately the same protein content, the degree of buffering could be correlated with loaf volume, and highly buffered flours produced small-volume loaves, despite high protein content.

Mangels [2] observed a "significant positive correlation between the protein content and baking strength of flour from hard red spring wheat in eight out of eleven crop years," but in addition found the quality of the gluten and the diastatic activity of the flour to be of importance. Later [3] he studied the water absorbing capacity of flour, but although there was a positive correlation between absorbing capacity of flour and protein content it was not marked. The degree of correlation between the water absorbing capacity of flour and loaf volume was also low.

From the experiments of James and Huber [4] it is evident that an important factor in flour quality is the force required to extend the gluten to breaking point. Only when this force was comparatively large could good loaf volumes be produced. High temperature (80°—120° C.) in air for 16 hours increased the breaking force of gluten. The gluten must act as a good binder for starch.

INFLUENCE OF STARCH IN FLOUR

That starch in wheat flour influences the strength of the flour has been the view of Jago,[5] Hardy,[6] Collatz,[7] Snyder [8] and others. Pure starch does not produce a good loaf since the dough is too short and not sufficiently elastic to hold gas.[9] Wo. Ostwald and Riedel [10] obtained satisfactory loaves by baking potato flour leavened with yeast and milk, the dough then being mixed with 15 per cent. by weight of a 30 per cent. starch paste. Bailey [11] reports a steady decrease in loaf volume and the texture as the starch content of bread is increased.

The size of starch grains in flours has been measured by Buchanan and Naudain [12] who differentiated between two classes of flours: (*a*) starch grains with diameters of 7 microns or less, (*b*) starch grains with diameters of 8 microns or more. The following table is compiled from their data:—

[1] *Vide* also *N. Zealand J. Sci. Tech.*, **8,** 299 (1926).
[2] *Cereal Chem.*, **3,** 150 (1926).
[3] *Ibid.*, **5,** 75 (1928).
[4] *Ibid.*, **4,** 449 (1927).
[5] "Technology of Breadmaking," p. 322.
[6] *Ibid.*, p. 319.
[7] Am. Inst. Baking, *Bull.*, **9.**
[8] U.S. Dept. Agr., *Bull.*, **101,** 56 (1901).
[9] Fornet, *Chem.-Ztg.*, **39,** 388 (1915).
[10] *Kolloid-Z.*, **17,** 12 (1915).
[11] "The Chemistry of Wheat Flour" (New York, 1925), p. 262.
[12] *Ind. Eng. Chem.*, **15,** 1050 (1923).

TABLE 29. SIZE OF STARCH GRAINS IN FLOURS.

Diameter of Starch Grains.	Flour Sample.						
	A	B	C	D	E	F	G
Microns.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
30	1·00	0·60	0·90	1·60	1·90	2·20	4·00
25—29	0·90	1·00	2·30	1·60	1·90	2·20	2·90
20—24	4·50	3·40	6·00	3·90	5·40	9·90	6·90
16—19	5·50	5·50	5·00	5·50	5·80	5·70	6·70
12—15	4·00	2·90	2·80	2·90	3·10	1·20	1·30
8—11	0·90	0·50	0·50	1·80	2·10	1·00	0·20
0—8	83·20	86·10	82·50	82·70	79·80	77·80	78·00
Average Diameter .	Microns 6·69	Microns 6·76	Microns 7·16	Microns 6·90	Microns 7·72	Microns 8·21	Microns 9·31
Loaf volume .	C.c. 1710	C.c. 1640	C.c. 1540	C.c. 1415	C.c. 1400	C.c. 1310	C.c. 1265

Strong flour was characterised by a high percentage of small starch grains, but it is difficult to say why. Possibly it is simply a reflection of the colloidal condition of the flour. On the other hand, Grewe and Bailey,[1] who measured the diameters of about 700 starch granules in each of seventeen samples of flour, could find no significant correlation between the size of starch granules and baking strength as reflected by the loaf volume.

Since starch *does* influence flour strength, the explanation for this must lie in another direction than mere size of the starch grains. Rask and Alsberg [2] in dealing with this problem, point out that the starch is inert until the grains are denatured by the baking temperature and such denaturation most probably occurs *after the loaf has formed* because of the coagulation of the proteins present. Hence, it is unlikely that there are differences in the starches sufficiently pronounced to exert a direct influence on the baking quality. On the other hand, the starch gel itself may play a *rôle* in the subsequent history of the loaf, *e.g.*, staling.

In a review of the subject, Alsberg [3] mentions that starches vary in composition and in degree of dispersion or colloidality. In dough, starch has two functions : (1) it is a source of fermentable carbohydrate produced by diastatic action, (2) it absorbs water, the

[1] *Cereal Chem.*, **4,** 244 (1927).
[2] *Ibid.*, **1,** 8 (1924).
[3] *Ibid.*, **4,** 485 (1927).

power of imbibition varying with different starches. The location of the starch in the flour is significant. In a strong flour the majority of the starch granules are firmly embedded in a gluten matrix, but in a weak flour, where the gluten masses are broken up, the starch grains are more accessible to the water used in preparing the dough. When a hard flour is overground numerous starch granules are freed from the matrix and the flour now resembles a soft flour, except that in the overground sample the starch grains are injured, whereby the swelling power with water is increased and diastase attack rendered much more rapid.[1] He finds that in bread the starch is not typically gelatinised, the starch grains being still intact but changed, since no dark cross is evident under the polarising microscope. The grains now take up Congo red, whereas normal starch grains do not.

Hall's work [2] confirms these observations, a microscope examination of hard spring wheat flour showing the starch grains to be embedded in a gluten matrix, whilst in soft winter wheat flour the aggregates of the starch grains are more loosely held in a smaller gluten matrix, and many free starch grains being evident. Hall points out that starch is particularly important in cake flours, since the dough is richer in water than bread doughs, *e.g.*, the latter have 60 per cent. added water based on 100 per cent. flour, whereas cake dough for layer cakes has 90—100 per cent. added water, and for jelly rolls, etc. the dough contains $>$ 100 per cent. added water. Batters are more the case than dough as usually conceived. "The very fact that corn starch may replace cake flour entirely in a loaf cake type batch and still produce that characteristic cake-like 'mealy' texture in the finished product, helps to strengthen our theory that the non-gluten constituents of cake flour are important." It was experimentally established that there is more complete gelatinisation of the starch in cake than in bread.

COLLOIDAL BEHAVIOUR OF DOUGH

In a very interesting essay Swanson [3] has summarised the question of the colloidal properties of dough. The basic idea is that dough being a mass of starch and protein particles covered by thin films of water, the colloid phenomena of dough centre round these particles. The degree of dispersion reached by the particles, their shape, and their internal and external form influence the colloidal behaviour. Undoubtedly complex aggregation, particularly of the protein particles, occurs and, therefore, dough has a network structure accompanied by an enormous interfacial area with water as the continuous phase.

[1] Alsberg and Griffing, *ibid.*, **2,** 325 (1925).
[2] *Ibid.*, **7,** 270 (1930).
[3] *Ibid.*, **2,** 265 (1925); **5,** 375 (1928).

The simplest conception of gluten formation in dough is that there is contact of the wetted protein aggregates to form a second continuous phase, viz., protein material in strands forming a net or mesh structure. The amount of water must not be too great, otherwise suspensions are formed. Incidentally, the rubbery nature of washed gluten or of dough is not exhibited by even a very stiff starch suspension. The latter, however, can be converted into a rubbery mass by addition of gluten.[1]

The double continuous phase system of dough contains starch grains and molecularly-dispersed ingredients like salt, sugar, and amino-acids. The addition of fat as a shortening agent (see p. 202) leads to adsorption of fat at the interfaces : protein-water and starch-water and weakens the adherence between particles. The quality of the dough is influenced by the number and nature of starch grains present and by the peculiarities of the protein resultant upon the biological genesis of the wheat kernel itself. Again, the hydration of the protein is influenced by the pH prevailing, and this, in turn, influences the important property of viscosity. The action of enzymes, too, modifies the structure of the protein aggregates, and is thus a factor in dough quality ; gluten from fermented dough is less tough and more easily stretched. The number of protein particles in a dough is important, for no matter how good the protein quality, too few particles cannot form a mesh of suitable strength. Again, the number of particles of protein is subsidiary to the quality of the protein. "The desired quality is obtained when the internal structure of the protein particles and the environment are such that strong strands are formed and when there are enough protein particles present to form enough such strands."

In a later paper, Swanson and Working [2] showed that it is possible to replace the usual fermentation of dough prior to panning by a mechanical action in a dough mixer, which packs and compresses the dough (gluten particles adhere) and pulls and tears the dough (gluten mixes with starch to form a porous network). Overworking the dough in this mixer results in a gluten which on washing out is granular and lacking in coherence and resiliency. Eventually, gluten cannot be washed out, as the starch and protein become so intimately mixed.

Several factors influence the quality of dough, *e.g.*, best baking results are given by flours ground to medium fineness.[3] The starch content is of great importance (as is discussed elsewhere, see p. 154), its admixture with flour decreasing the extensibility of dough.[4] The

[1] Olson, Wash. Agr. Expt. Sta., *Bull.*, **144,** 5 (1917).
[2] *Cereal Chem.*, **3,** 65 (1926).
[3] Van der Lee, *Chem. Weekblad.*, **25,** 34 (1928) ; Schollenberger and Coleman, U.S. Dept. Agr., *Bull.*, **1463,** 1—35 (1926) ; Le Clerc *et al.*, *Operative Miller*, **24,** 257 (1919).
[4] Cf. Bailey and Le Vesconte, *Cereal Chem.*, **1,** 61 (1924).

extensibility is a maximum at pH 6, with a marked reduction at <pH 5 or> pH 7 ; swelling and syneresis phenomena are here involved with the gluten content.[1]

The " viscosity " of dough at 30° C. has been found [2] to be a minimum at pH 4·75, this reaction, therefore, being the most favourable for the rise of dough. Several investigators agree that pH 5 is the ideal reaction for bread-making.[3] Salt in dough at first lowers the viscosity and with further concentration increases it. The minimum viscosity occurs with at 0·12 mol. NaCl concentration. In the presence of acid, the effect of NaCl varies with the pH of the system, the greater the acidity the more marked being the viscosity lowering influence of the salt. The presence of salt gives a tougher and stiffer dough and without salt the bread is dull and very open.[4]

INFLUENCE OF ACIDITY

Harrel [5] exposed gluten to the action of acid and then added it to dough, noting a large increase in the volume of the subsequent loaf. Accordingly he classified the ingredients of dough : (1) resisting increase in acid value (water, flour, milk, malt, salts), *i.e.*, having a buffer action ; (2) those tending to overcome buffering (yeast and acid accelerating salts).

Although the earlier work seemed to connect an optimum acidity solely with the desirable physical condition of the dough protein and the course of its denaturation by heat,[6] it is becoming more and more recognised that any improvement in the physical properties of the dough is due to the accelerated activity of the enzymes present, particularly diastase.[7]

Fisher and Halton [8] point out that the pH of a loaf is about 0·2—0·3 higher than the pH of the original dough. They do not attribute that importance to pH value that seems so usual and their extensive series of baking tests over a wide pH range (7·8—4·2) with varying periods of fermentation confirmed their view that " H-ion concentration is not a factor of great importance in determining dough or loaf quality. Alterations of H-ion concentration brought about in doughs by additions of acid several times greater than those brought about by prolonged over-fermentation appear to have singularly little effect on dough or loaf characters." Again, the pH effects are not always observed in the same direction.

[1] Cf. Brownlee and Bailey, *ibid.*, **7,** 487 (1930).
[2] Henderson, Fenn and Cohn, *J. Gen. Physiol.*, **1,** 387 (1919) ; Cohn and Henderson, *Science*, **48,** 501 (1918).
[3] Jessen-Hansen, *Compt. rend.*, **10,** 170 (1911) ; d'Arbouet, *Chimie et Industrie* **14,** 21 (1925) and *Rev. gén. Coll.*, **4,** 199, 225 (1926) ; Schröder, *Arb. Reichsgesundh* **57,** 598 (1916) ; Sørensen, *Am. Food. J.*, **19,** 556 (1925).
[4] Harrel, *Cereal Chem.*, **4,** 425 (1927).
[5] *Ibid.*, **3,** 1 (1926).
[6] *Vide* Sørensen, *Am. Food J.*, **19,** 556 (1924).
[7] Grewe and Bailey, *Cereal Chem.*, **4,** 269 (1927).
[8] *Ibid.*, **6,** 32, 97 (1929).

Certain common effects, however, are increased toughness of the dough, a more pronounced flavour and an improved crumb colour. Harrel [1] also finds a whiter bread as the pH is lowered, and believes this is due to increased dispersion of the gluten. He, too, states that one must not be obsessed with the idea of a pH 5 optimum, since the acidity required varies with the baker's requirements in loaf volume and flavour.

TENACITY OF DOUGH

An apparatus has been described by Chopin [2] for measuring the tenacity of dough and its ability to stretch into a membrane. The factors influencing such dough properties are temperature, recipe, method of working the dough, age of the flour, and age of the dough. He found that the difference between the specific volume of the

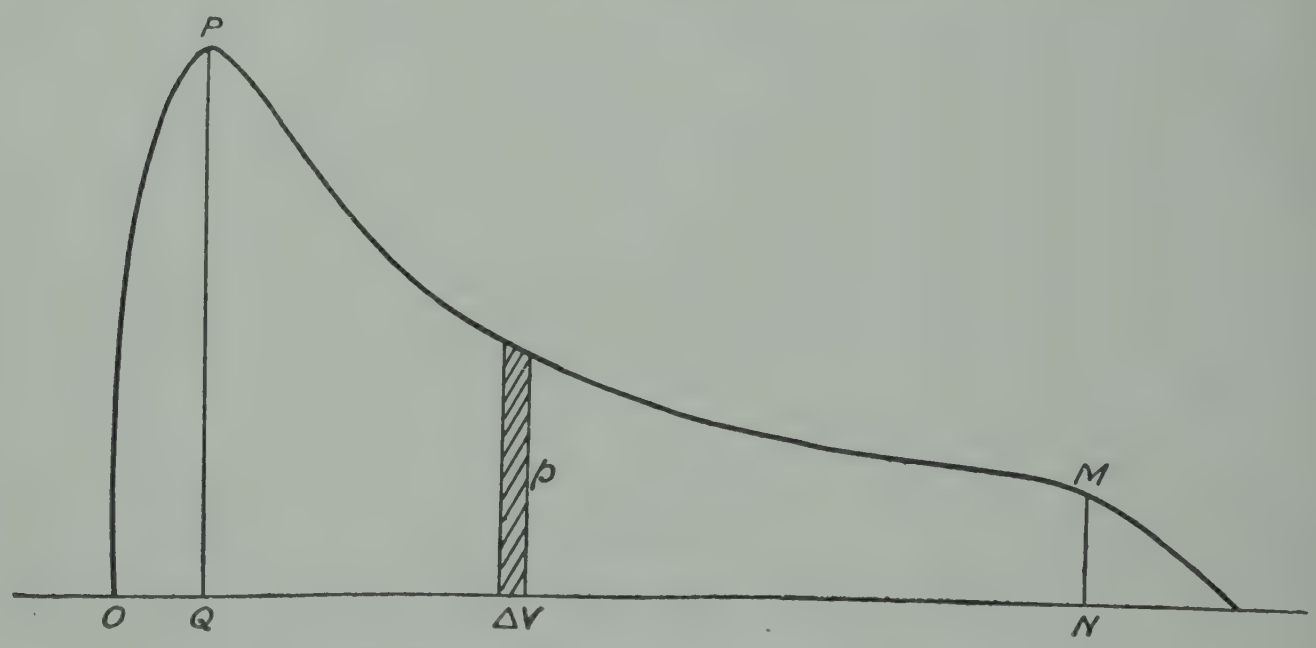


FIG. 17.—Typical curve traced by recording pressure gauge.

bread and the original specific volume of the dough employed varies as the square root of the coefficient of extension of the dough as a thin membrane.

In the test, the sample of dough swells to a spherical bubble, which increases in size and stretches to a thin membrane, eventually weakening and bursting, the while a recording device traces out the air pressure. Fig. 17, reproduced from Chopin's paper, shows the general type of curve obtained. At the maximum pressure, the ordinate PQ represents the tensile strength of the dough in its original condition, and the ordinate MN the tensile strength at the time of rupture, *i.e.*, as the thinnest membrane possible.

The curve varies widely with the type of wheat from which the flour is milled as well as the method of milling. The plastic proper-

[1] *Ibid.*, **4,** 434 (1927); *vide* also St. John, *ibid.*, **6,** 409 (1929).

[2] *Bull. soc. encour. ind. natl.*, **133,** 261 (1921); *Cereal Chem.*, **4,** 1 (1927). Cf. Bailey and Le Vesconte, *ibid.*, **1,** 38 (1924) and Engledow, *ibid.*, **5,** 1 (1928); Harrel, *ibid.*, **4,** 283 (1927); Kuhn and Richter, *Kolloidchem. Beihefte*, **22,** 445 (1926); Kress, *Cereal Chem.*, **1,** 247 (1924); Grewe and Bailey, *ibid.*, **4,** 239 (1927).

ties of a dough are defined by the energy required to stretch the dough and the coefficient of extension.

ACTION OF HEAT ON GLUTEN

Heating organic colloids usually brings about new physico-chemical behaviour due to an alteration in the degree of dispersity, and frequently denaturation or coagulation phenomena are seen. Very litle work has been done in this connection with gluten, but preliminary investigations by Alsberg and Griffing [1] have shown that gluten swells less in acid after being heated from 50°—80° C. There is a slight increase in swelling power over the temperature range, 30°—50° C., but from 50°—80° C. denaturation takes place. Obviously, such physico-chemical changes are important in baking, and suggest a field for interesting research. Since exposure of gluten to moist heat at 80° C. for 30 minutes still leaves it with the capacity to swell in acid, the authors point out that " in bread it has not lost all its colloidal properties as competely as some have assumed. It may play an important *rôle* in the growing stale of bread."

THE STALING OF BREAD

The phenomenon of the staling of bread offers a problem of extraordinary interest to colloid chemists. Earlier workers believed that as bread ages, the " fixed " water held by the starch in colloidal solution in the crumb, leaves the crumb and is taken up by the crust.[2] Boussingault [3] observed that heating the crumb to 70° C. rendered stale bread fresh again. Bibra confirmed this observation, but found that if the water content is less than 30 per cent. heat does not refreshen stale bread, whilst if the bread be dipped in water for a few seconds and then heated to 80° C. it becomes fresh. In his opinion, staleness is due to water combining with starch and gluten, the water in fresh bread being uncombined.

The most extensive investigations in this field have been carried out by Katz, who has recently provided a summary of his valuable Dutch publications of the years 1912—1916.[4] This summary provides a good starting-point for the student wishing to follow up the subsequent literature.

In 1912 Katz and Verschaffelt held the opinion that staling arose from an alteration of the starch (which had been partly gelatinised during baking) leading to a diminished capacity to hold water. Thus the equilibrium between starch and the gluten skeleton is altered, the gluten absorbing some of the water previously held by

[1] *Cereal Chem.*, **4**, 411 (1927).
[2] Birmbraum, " Das Brotbacken " (Braunschweig, 1878), p. 255.
[3] *Ann. chim. phys.*, (3), **36**, 490 (1853).
[4] Walton's " A Comprehensive Survey of the Chemistry of Starch," Vol. I., 100 *seq.*

the starch. Due to the shrinkage of the starch granules and the relaxation of their union with the gluten skeleton, the stale bread crumbles. Hardening of the crumb also occurs, the nature of the bread deciding whether crumbling or hardening predominates. It is important to note that both changes can occur even when conditions provide for no loss of water on the part of the whole bread. The authors found that under the microscope, the starch granules in stale bread have a sharper and double contour, a fine air bubble occurring between the two contours.

Katz observed that if bread is not cooled, staling does not occur. At a temperature of 60° C. or more, under constant humidity conditions, staling could be indefinitely delayed. Since bread crumb can be "refreshened" by heat and this process repeated several times until the bread loses too much moisture, Katz postulates that there is a physico-chemical equilibrium between fresh and stale bread. It was experimentally demonstrated that "the degree of staling of a piece of bread crumb kept for 24—48 hours may be represented graphically by a continuous curve as a function of the temperature. There is no sharp break or transition point and, apparently, a homogeneous state of equilibrium prevails." In 24—48 hours, at 60° C. or more, the crumb remains fresh, at 40° C. it becomes approximately half-stale, at 30° C. this is intensified and at 17° C. or 0° C. staleness becomes complete. It was shown that with intense cold (—185° C) the crumb was quite fresh even after 48 hours; maximum staling occurs at —2° to — 3° C.

The crust of bread stales due to water absorption and chemical changes cannot be detected. Maintaining bread in an air current of humidity of about 65—75 per cent. prevents this staling, the water which diffuses from the crumb to the crust being regularly removed.[1]

To sum up, staling of crumb is accompanied by crumbling, hardening, loss in swelling capacity and diminution in content of soluble starch, the latter in 48 hours being approximately 45 per cent. of the original value. Whilst hardness, swelling capacity and content of soluble starch reach stale bread values in 9—10 hours, it takes 24 hours for crumbling to attain and "this difference in time is dependent upon the time required for water to diffuse from the starch to the gluten."

If excess of water is present, staling is indefinitely inhibited. Also, traces of aldehydes inhibit staling to a remarkable extent, as do also strongly basic substances. Pyridine and dipropylamine are very active, chloroform and allyl alcohol less so. Just what is the function of these compounds in restraining staling is in dispute. It would seem that a study of their influence on starch hydrogel and gluten hydrogel is required to elucidate their action.

[1] See Katz, Brit. Pat., 5154 (1914)—to maintain bread in the fresh state, hold it in an air current of about 85 per cent. humidity.

Katz has discussed the relation between the staling of bread and the retrogradation of starch paste. Starch paste stales and undergoes those changes associated with staling of bread crumb, except that no crumbling occurs, and this is true of starches in general. The starch which separates out from a starch solution on ageing is termed "retrograded starch," and the phenomenon "retrogradation." He distinguishes between two grades of gelatinisation of starch :—

(i.) "Gelatinisation of the first order" is the preliminary swelling of starch granules on heating in water, the starch still remaining a white powder.

(ii.) "Gelatinisation of the second order" occurs when the whole mass changes to that characteristic condition of the swollen granules, resulting in a transparent, opalescent paste.

Grade (i.) arises when starch is heated to 60°—70° C. with excess water or when air-dried starch is homogeneously saturated with 45 per cent. water and heated in a closed tube to 100° C. Grade (ii.) obtains when starch and sufficient water are heated to 100° C.

Neither in the first nor second order of gelatinisation does retrogradation of starch occur when preserved at 60° C. or higher, this pointing to the fact that gelatinisation is an equilibrium process. In other words, gelatinisation of starch is a chemical process and not a physical one, a notion supported by the study of the X-ray spectrum of starch.[1] Katz showed that the X-ray diagram of starch remains unchanged when the starch swells or contracts, *i.e.*, takes up or loses hygroscopic water, but a marked change occurs during gelatinisation, the X-ray spectrogram pointing to a less crystalline condition. "*Through retrogradation*, an extremely interesting change occurs in the X-ray spectrogram of starch : *The interferences of the non-gelatinised starch come back ;* those of gelatinised starch remain visible. We therefore see that X-ray spectrography too shows this process to be a 'retrogradation,' *i.e.*, a returning of gelatinised starch to the ungelatinised condition."

Neumann [2] agrees that the staling of bread is due to the influence of the loss of water on the colloidal system : starch/water, and that the water lost is partly evaporated and partly absorbed by the protein present. Staleness of bread is thus due to the progressive separation of amorphous starch. He points out the need for research on the relation to staling of the physical and chemical nature of the starch grains, of the ash content of the flour, and of the salt in the dough. Bryant [3] agrees that staleness is due to soluble starch reverting to the insoluble form.

[1] Walton's "A Comprehensive Survey of the Chemistry of Starch," Vol. I., 74 (New York, 1928) ; *Z. physik. Chem.*, **150,** 37 (1930) ; Katz and Rientsma, *ibid.*, **150,** 60, 67 (1930).

[2] *Z. ges. Getreidew.*, **6,** 119 (1914).

[3] *Northwestern Miller*, **147,** 747 (1926).

Wo. Ostwald [1] definitely considers staling as due to syneresis of starch gel, *i.e.*, the squeezing out of water, and rejects the physico-chemical equilibrium theory of Katz. Barnard and Bishop [2] observed that wrapping bread in semiporous waxed or paraffin paper retards loss of moisture and inhibits staleness up to three days. The moisture of the crumb is *not* imparted to the crust, " a loss of the moisture content of the crumb is accompanied by similar and almost exactly parallel loss of the moisture content of the crust." Hence wrapping " tends to the preservation of the colloidal condition and physico-chemical equilibrium noted by Katz, the destruction of which results in staleness."

Alsberg [3] has criticised the Katz hypothesis of a physico-chemical equilibrium of starch involved in bread-staling, pointing out that Katz is not clear whether his so-called retrogradation is to be considered as syneresis. Alsberg aptly reminds us that we are ignorant as to whether retrogradation or syneresis occurs at all in starch in the concentrated form in which it occurs in bread. Although staleness is reversible through heat, starch retrogradation is not, and further, in concentrated starch systems, such as bread represents, retrogradation of starch is slow, whereas the staling of bread crumb can be rapid.

Accordingly, Alsberg believes that, important though the changes in starch may be, it is possible that changes in the colloidal properties of gluten may be one of the factors in the phenomenon of staling. That gluten is not an inert coagulum in bread is proved by work in Alsberg's laboratory showing that gluten heated to as high a temperature as 80° C. is still able to swell appreciably in weak acid.[4]

Whymper [5] also emphasises the importance of gluten in the staling phenomenon, suggesting that gluten acts as a protective colloid to starch in solution. He found that addition of extra gluten to flour-dough improved the keeping qualities of the resulting bread. No quantitative research was made on this question, and Whymper summarised his views, attributing staleness to : (*a*) deposition of solid starch in the crumb, commencing between 6 and 24 hours cooling period ; (*b*) partial polymerisation of starch, independent of the deposition already stated, which tends to crumble the gelatinous nature of the bread-crumb when fresh.

Karácsony [6] investigated the *rôle* of protein and carbohydrate in staling, finding no significant difference in the solubility of the gluten of fresh bread and 48 hours old bread. He concluded : " It can be

[1] *Kolloid-Z.*, **25,** 36 (1919).

[2] *Ind. Eng. Chem.*, **6,** 736 (1914). *Vide* also Sadtler, " Report on Bread Wrapping," *Proc. Nat. Assoc. Master Bakers*, **16,** 161—93 (1915).

[3] Walton's " A Comprehensive Survey of the Chemistry of Starch." Vol. I., 95 (1928) ; *Cereal Chem.*, **4,** 489 (1927).

[4] Alsberg and Griffing, *Cereal Chem.*, **4,** 421 (1927).

[5] *Brit. Assocn. Colloid Repts.*, **3,** 64 (1920).

[6] *Z. Untersuch. Lebensm.*, **56,** 479 (1928).

accepted as proved that during staling the water-soluble carbohydrate decreases, and this is best explained on the basis of syneresis of the starch gel." This worker [1] has shown the interesting fact that the pH value of bread crumb kept at room temperature does not change or only slightly increases, thus supporting Lehmann's conclusion that the acidity of bread kept in a covered vessel remains constant.[2] Recently Karácsony [3] has published data on the viscosity of bread crumb suspensions, finely sieved, and shown that the percentage decrease in viscosity of a bread after 48 hours staling is of the order 32—37 per cent. of the viscosity of the fresh bread. Should these results be confirmed and extended, the method would provide a rapid and simple means of following the staling of bread.

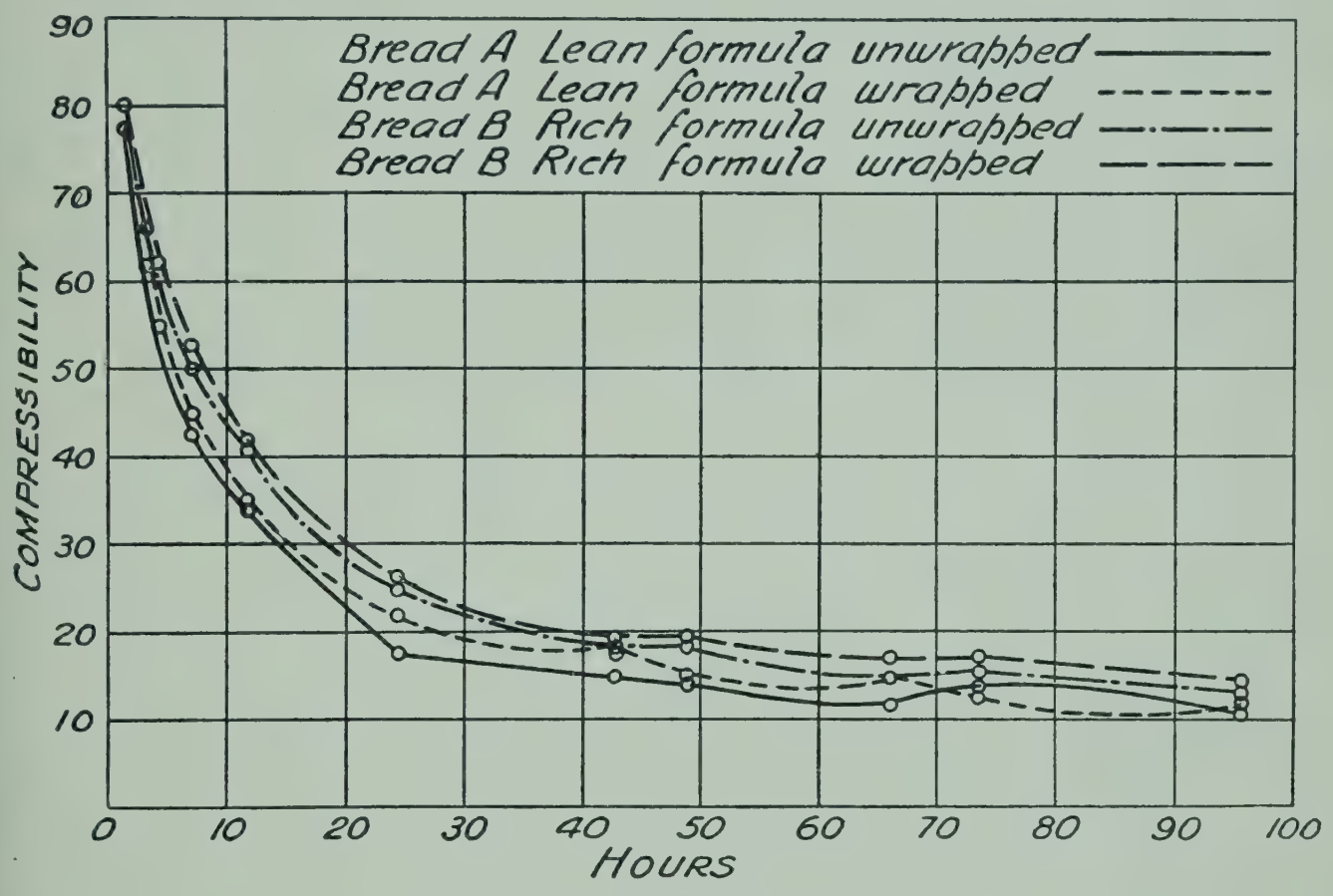


FIG. 18.—Typical compressibility curves.

In a recent review of the phenomenon of staling, Platt [4] points out that apart from the loss of volatile constituents ("evaporation losses") and possible changes due to oxidation, there is an "inherent staling," *i.e.*, "changes that take place apart from losses or gains from the surroundings." He devised a method for following staling by determining the compressibility of bread crumb. When bread is stored under ordinary commercial conditions where the moisture loss is usually about 4·7 per cent., or when it is stored so that moisture is not lost, the compressibility-time curve is characteristic. Fig. 18 reproduces some of the author's graphs. Platt emphasises that the determination of compressibility *as such* does not measure staleness,

[1] *Cereal Chem.*, **5**, 477 (1928).
[2] *Arch. Hyg.*, **44**, 214 (1902). Cf. also Barnard and Bishop, *Ind. Eng. Chem.*, **6**, 736 (1914).
[3] *Cereal Chem.*, **6**, 241 (1929).
[4] *Ibid.*, **7**, 1 (1930).

but that "inherent staleness may be indicated as the *change* in compressibility taking as the starting point the compressibility of the crumb as soon after baking as it has reached room temperature, (say 2 hours), and measuring the change in this initial compressibility after a given period of ageing." The slope of the tangent to the compressibility-time curve, *i.e.*, the rate of change of compressibility, measures the *rate* of inherent staling.

CHAPTER VI

EMULSIONS

An emulsion is a system of two liquids, one being dispersed as globules in the other. The liquid which is sub-divided, is termed the internal or dispersed phase, the other liquid being the external or continuous phase. With two liquids, A and B, emulsions are possible of A dispersed in B, and *vice versa*. Usually the liquids are an oil and an aqueous solution, the emulsions being of the type oil-in-water or water-in-oil, depending on the order in which the liquids are the internal and external phases respectively.

Pure oils and pure water alone form the oil-in-water type of emulsion, and even then the concentration of the oil is only of the order of 0·0001 per cent. Such emulsions or oil-hydrosols are of great importance in theoretical colloid physics, but have little bearing in food technology. The more concentrated emulsions familiar in industrial practice, are only possible when a third phase is present, *viz.*, the emulsifier or emulsifying agent.

The principal edible emulsions are milk, cream, butter and margarine (really solidified emulsions), salad cream, mayonnaise, and pharmaceutical emulsions. The preparation and properties of these emulsions present very interesting problems in colloid physics and chemistry and involve a sound knowledge of the modern theories concerning emulsion systems in general.

The present writer has presented elsewhere [1] a detailed survey and discussion of the phenomena and theories relating to emulsions and emulsification. For the present volume this detailed account may be summarised briefly, followed by a more specialised treatment of the subjects concerned.

Food emulsions are invariably of the oil-in-water (O/W) type. Margarine *as a solid* may happen to be of the opposite type (W/O), but as a true emulsion in the course of manufacture, it is usually an O/W system.

The type of emulsion promoted by the emulsifying agent depends on the nature of this agent, water-soluble colloids promoting the dispersion of oil in water, and oil-soluble colloids promoting the dispersion of water in oil. The most important colloid emulsifying agents used in the preparation of edible emulsions are : gelatin,[2]

[1] Clayton, "The Theory of Emulsions and Their Technical Treatment" (London, 1928).

[2] Cf. Zhukov and Bushmakin, *J. Russ. Phys. Chem. Soc.*, **59,** 1061 (1927) ; Kernot and Knaggs, *J. Soc. Chem. Ind.*, **47,** 96 T (1928).

egg-yolk, pectin, gum arabic, gum acacia, gum tragacanth, locust kernel gum, casein, starch, Irish moss, agar-agar [1] and albumins. All promote the formation of O/W type of emulsions. Solids, too, are able to act as emulsifying agents, the continuous phase being that liquid which the more readily wets the solids.[2]

Under suitable conditions, to be referred to later, emulsions may be produced of any desired concentration. Pickering [3] was able to disperse 99 c.c. of oil in 1 c.c. of 1 per cent. aqueous solution of potash soap, whilst Briggs and Schmidt [4] prepared an emulsion containing over 90 per cent. of water in benzene.

The number of possible emulsifying agents leads naturally to the question of their efficiency in promoting and stabilising emulsions. Unfortunately, no systematic attempt has been made to arrange all the emulsifying agents in the order of their capacity in this respect. Thus, it is only known in a general kind of way that certain substances are better emulsifiers than others for emulsions of, for example, olive oil in water. The position is complicated, too, because the order of efficiency for promoting and maintaining emulsions of oil in water would undoubtedly require re-arrangement when the water contained electrolytes such as sodium chloride or lactic or acetic acid. Any change in the character of the emulsifying agent due to the presence of solutes in the water would at once be reflected in the degree of dispersion or the stability of the emulsion. Thus, the investigations of Limburg [5] and Zhukov and Bushmakin [6] point to the alteration of emulsifying capacity of gelatin with change in the hydrogen ion concentration of the aqueous phase (see p. 70). Agar-agar or starch would not be so sensitive in this respect.

Suggestive is the work of Clark and Mann,[7] who studied the efficiencies of sucrose, dextrin, starch, gum arabic and egg albumin. Emulsions of benzene and of kerosene were made in aqueous solutions of the emulsifying agents at various concentrations. In each instance the oil phase was 75 per cent. of the total volume. After seven weeks the emulsions were graded on an arbitrary scale of ten as regards stability, *i.e.*, efficiency of the emulsifying agents.

Thus, using 1 per cent. solutions of the emulsifiers and dispersing benzene therein, the following gradation was given : egg albumin, 10 ; starch, 6 ; gum arabic, 5 ; dextrin, 3 ; sucrose, 0. When kerosene was emulsified, the gradation appeared as : egg albumin, 10 ; starch, 9 ; gum arabic, 3 ; dextrin, 2 ; sucrose, 2. The presence of electrolytes influenced the emulsifying efficiencies, but egg albumin still stood out as the most effective, and the emulsions

[1] Turner, *J. Infectious Diseases*, **44,** 134 (1929).
[2] *Vide* Smith, *Phil. Mag.* (7), **4,** 820 (1927).
[3] *J. Chem. Soc.*, **91,** 2002 (1907).
[4] *J. Phys. Chem.*, **19,** 491 (1915).
[5] *Rec. trav. chim.*, **45,** 885 (1926).
[6] *Loc. cit.*
[7] *J. Biol. Chem.*, **52,** 160 (1922).

still received 10 points on the arbitrary scale when a variety of inorganic electrolytes was used in aqueous solution.

The relative emulsifying efficiencies of gum arabic, gelatin, agar and gum tragacanth were investigated by Whitmore and Linehan [1] working with emulsions, always containing 5 per cent. by volume of oil of orange. They reduced the amount of colloid until the emulsion broke when centrifuged, and thus determined the minimal amount of colloid necessary to stabilise the emulsion under such conditions.

The minimal amounts for gum arabic, gelatin, agar and gum tragacanth were (in per cent.) 0·5, 0·05, 0·025 and 0·5 respectively, and the corresponding emulsions remained stable for 21, 28, 3 and 28 days respectively.

According to Krantz and Gordon [2] the size of globules of vegetable and mineral oils in emulsions stabilised with gum acacia is smaller and far more uniform than in similar emulsions employing gum tragacanth. The acacia emulsions were stable over the range pH 2-10, the tragacanth emulsions over the range pH 1·9—2·3. Contrary to Krantz and Gordon's results are the findings of Crockett and Oesper [3] and of Hiss.[4]

Some interesting results regarding mixed emulsifying agents in the preparation of pharmaceutical emulsions were obtained by Smith and Hazley.[5] A mixture of gum acacia and gum tragacanth proved more efficient than either gum alone. Gum acacia gave good emulsions, but their low viscosity permitted creaming. Gum tragacanth gave coarse emulsions which did not break as the mucilage possessed a high viscosity. Hence in the mixture of gums, the excellent dispersive property of gum acacia is aided by the mechanical resistance to creaming imposed by the presence of the gum tragacanth.

When apple pectin was used with gum acacia the emulsions showed clear water separation below on standing. This is considered due to the setting of the pectin to a gel and subsequent syneresis. Similarly, a mixture of pectin and agar gave emulsions which creamed rapidly.

A splendid field of research is open to the technical colloid chemist, who should investigate the ability of various emulsifying agents to stabilise food emulsions under the required conditions imposed by the aqueous phase of the emulsion food in question. The mechanical factors (order of mixing and degree of agitation) and temperature should be constant [6] and the amount of oil capable

[1] *Ind. Eng. Chem.*, **21,** 879 (1929).
[2] *J. Am. Pharm. Assoc.*, **15,** 93 (1926); Krantz, *ibid.*, **19,** 1181 (1930).
[3] *Ind. Eng. Chem.*, **9,** 967 (1917).
[4] *Bull. sci. pharmacol.*, **13,** 229 (1899).
[5] *Quart. J. Pharm. Pharmacol.*, **3,** 362 (1930).
[6] Clayton, *J. Soc. Chem. Ind.*, **45,** 288 T (1926); Smith, *ibid.*, **46,** 345 T (1927).

of being held in the emulsion state should be correlated with the degree of dispersion attained, *i.e.*, a size-frequency analysis [1] should be made on the emulsion.

THE CONCENTRATION OF EMULSIONS

We have observed that emulsions of any desired concentration are possible under suitable conditions. This is only the case when the emulsion globules are not uniform in size. If into a given space small spheres of the same diameter are packed as closely as possible, the volume they will occupy is 74·048 per cent. of the total available volume, and this figure is independent of the size of the globules, each of which is touched by twelve other globules.[2] However, in emulsions as usually prepared, the globules are of unequal diameters, thus permitting packing of smaller globules between the larger. A larger volume percentage can thus be occupied. Mathematically, such packing into the interstices with smaller and smaller spheres is a variable function approaching, but not reaching, 100 per cent. as a limit.

Another point to note is that coalescence of globules does not necessarily occur when the emulsion globules touch. The presence of a protective film (of the emulsifying agent) round the globules permits squeezing together until a vanishingly small space separates them. Deformed globules are quite usual in concentrated emulsions.

Very frequently, emulsions which are not very stable will "cream," *i.e.*, a more stable emulsion rises above the system. Cream on milk is a familiar example. The "cream emulsion" will often be found on analysis to have a concentration of the dispersed phase of the order of 74 per cent. by volume. Woodman [3] has investigated this phenomenon, preparing emulsions of toluene, benzene and paraffin in 0·4 per cent. gelatin solution. The percentage of oil taken to the total volume of both phases varied from 0·7 to 83·3 per cent. in thirty-four different experiments. On standing, the emulsions creamed, and analysis of the creams revealed that they contained on the average 72·28 per cent. by volume of the oil, the actual range being 65·5 to 80·5 per cent. The emulsions containing initially from 75 to 83·3 per cent. oil did not yield creams of greater oil content. Woodman argues "that there is only one *true* emulsion which can exist permanently, the emulsion being one containing 74·048 per cent. of internal phase by volume." Less concentrated emulsions are considered as mixtures of this true emulsion and excess of external phase ; more concentrated emulsions

[1] Smith and Grinling, *Quart. J. Pharm. Pharmacol.*, **3,** 354 (1930) ; Davey, *J. Phys. Chem.*, **35** 115 (1931).

[2] Clayton, "The Theory of Emulsions and Their Technical Treatment" (London, 1928), p. **45** ; Wo. Ostwald, *Kolloid-Z.*, **47,** 131 (1929).

[3] *J. Pomology Hort. Sci.*, **4,** 184 (1925).

arise because the oil globules are not uniform in size and are deformable.

Woodman's suggestion is certainly interesting and probably concerns an ideal theoretical emulsion. However, the fact is that with sufficient of the emulsifying agent, the proper order of the mixing of the liquids and the correct conditions of agitation, stable emulsions of any concentration are possible. These conditions will be elaborated later.

THEORIES OF EMULSIFICATION

Three theories concerning the formation of emulsions require examination : (*a*) the viscosity theory, (*b*) the hydration theory, (*c*) the surface tension and adsorption theory.

The Viscosity Theory. The viscosity of the continuous phase has an important bearing on the stability of an emulsion, a fact recognised by such early workers as Hillyer,[1] Marshall [2] and Pickering.[3] The majority of investigators to-day accept the conclusion that *viscosity aids emulsification solely by virtue of the hindrance offered to coalescence of the dispersed globules*, and is not the *cause* of emulsification.[4]

The work of Holmes and Child [5] on gelatin as an emulsifying agent led these authors to the conclusion that "the leading factor in oil-water emulsification with the aid of gelatin is viscosity, not the maximum, but the most favourable viscosity." The present writer has disputed this claim, whilst Krantz and Gordon [6] also assert that viscosity is not the *sine quâ non* factor in emulsification. Clark and Mann,[7] too, in a study of kerosene emulsions, conclude : "viscosity cannot be considered the only prime requisite of an emulsifying agent."

According to the recent work of Canals and Mousseron [8] the emulsifying efficiencies of gum arabic and gum tragacanth for emulsions of olive oil and sweet almond oil in water, vary with the viscosity of the system. Taking the viscosity of water as unity, gum tragacanth was more efficient than gum arabic when the viscosities were high (16·0 and 15·4 respectively), but at low viscosities (7·1 and 9·2 respectively) gum arabic was the more efficient agent. Increasing the viscosity by addition of glycerol reduced the emulsifying efficiency of gum tragacanth but increased that of gum arabic.

The Hydration Theory. According to this theory, advocated by

[1] *J. Am. Chem. Soc.*, **25,** 513 (1903).
[2] *Pharm. J.*, **28,** 264 (1909).
[3] *J. Chem. Soc.*, **91,** 2001 (1907) ; *vide* Smith, *Phil. Mag.*, (7), **4,** 820 (1927).
[4] Cf. Whitmore and Linehan, *Ind. Eng. Chem.*, **21,** 879 (1929).
[5] *J. Am. Chem. Soc.*, **42,** 2049 (1920).
[6] *J. Am. Pharm. Assoc.*, **15,** 93 (1926).
[7] *J. Biol. Chem.* **52,** 157 (1922).
[8] *Bull. Sci. Pharmacol.*, **33,** 283 (1926).

Fischer,[1] emulsions can only be produced when the liquid which is to become the external phase is all used in the formation of a hydrated compound of the emulsifying agent employed. On this view, for example, " when it is said that the addition of soap favours the formation and stabilisation of a division of oil in water, it really means that soap is a hydrophile colloid which, with water, forms a colloid hydrate with certain physical characteristics, and that the oil is divided in this. The resulting mixture cannot, therefore, be looked upon as a sub-division of oil in water, but rather as one of oil in a hydrated colloid."

Emulsifying efficiency is thus considered to be intimately related to the degree of hydration of gelatin, starch, agar-agar or other emulsifying agent. However, no extension of the theory has been made to oil-soluble colloids and their promotion of W/O emulsions. The present writer [2] has criticised Fischer's theory in some detail, summing up the position as follows : " The essential difference between Fischer's theory and the present theory of an adsorbed film enveloping the emulsion globules is that in the former case the emulsifying agent is supposed to be evenly distributed throughout the aqueous phase as some sort of chemical compound with the water ; the oil globules are dispersed in this aqueous phase because of its viscosity or " covering power." The modern theory believes that a *concentration* of the emulsifying agent occurs at the interface between the oil globules and the aqueous phase. Strangely enough, this very idea of adsorbed film protection of emulsion particles has to be resorted to by Fischer [3] in order to explain the formation of emulsions with finely-divided solids as emulsifying agents, and also to account for the stability of milk when diluted with water."

The Surface Tension Theory. When two immiscible liquids are in contact the boundary surface between them is referred to as the liquid/liquid, or the dineric, interface. This is not an absolutely distinct line of demarcation, but rather a very attenuated layer (a few millionths of a centimetre deep), due to the interpenetration of both phases. In emulsions, whether of the O/W or the W/O type, there exists a relatively large interfacial separating layer, the area of which increases very rapidly with increasing subdivision of the internal phase.

The surface tension at the boundary between two immiscible liquids is termed the interfacial tension. It can be shown on thermodynamical grounds that, if a third substance now be added which will affect the value of the interfacial tension, concentration phenomena appear, *i.e.*, the concentration of the added substance will be different in the main bulk of the continuous phase to what it

[1] Fischer and Hooker, " Fats and Fatty Degeneration " (New York, 1917), p. 5.
[2] *Vide* Clayton, *loc. cit.*, p. 61.
[3] *Loc. cit.*, p. 53.

is at the interfacial separating surface. The most stable arrangement accompanying interfaces in general is that the surface energy should be a minimum. Consequently, if a dissolved substance raises the potential energy at the interface, it will tend to leave that interface; if it diminishes the potential energy, then it will concentrate as the interface.

The famous Willard Gibbs-Thomson equation [1] concerning changes in concentration at the interface is :—

$$\Gamma = -\frac{c}{\mathrm{RT}} \cdot \frac{d\sigma}{dc}.$$

Where Γ = the surface excess concentration of the solute.
c = concentration of the solute in the bulk of the solution.
σ = surface or interfacial tension.

Since $\frac{d\sigma}{dc}$ is the differential co-efficient of the function connecting interfacial tension and concentration, it follows that $\frac{d\sigma}{dc}$ is positive when the solute increases the interfacial tension, and negative when it decreases it. Consequently, Γ is positive when the solute lowers the interfacial tension, *i.e.*, the added substance will be adsorbed at a dineric interface if by its adsorption the interfacial tension is reduced (see p. 389).

The work of Donnan [2] proved conclusively the relation between lowering of interfacial tension and capacity for emulsification, when a third agent was dissolved in the aqueous phase, an oil being the internal phase. Donnan worked mainly with soaps and did not investigate the case where a solute in the oil phase would also lower the interfacial tension between oil and water.

Ramsden,[3] later, demonstrated in the case of several stable emulsions, actual solid membrane surrounding the oil globules when olive oil was emulsified in aqueous saponin solutions. He concluded that "the persistence of many emulsions is determined largely, among other factors, by the presence of solid or highly viscous matter at the interfaces of the two liquids," and that "accumulation of solid matter at the interfaces of the above emulsion—pairs occurs because the surface energy is thereby diminished."

Many investigators [4] have reported visualising enveloping films around the globules of oil or water in various emulsions. There can now be no doubt that an emulsifying agent does concentrate at the dineric interface, and sometimes the act of adsorption leads to a

[1] *Vide* Clayton, *loc. cit.*, p. 72 ; Bancroft and Tucker, *J. Phys. Chem.*, **31,** 1681 (1927).
[2] *Z. physik. Chem.*, **31,** 42 (1899) ; *Kolloid-Z.*, **4,** 208 (1910).
[3] *Proc. Roy. Soc.*, (A), **72,** 156 (1903) ; *Z. physik. Chem.*, **47,** 336 (1904).
[4] *Vide* Clayton, *loc. cit.*, p. 75.

change in the physical character of the emulsifying agent, this being "precipitated" as a fibrous or membrano-fibrous solid, no longer soluble in its original solvent.

It is unnecessary to pursue the question of solid powders as emulsifying agents, except to state that the type of emulsion promoted is such that the external phase is that liquid which is the better wetting agent for the solid. The use of solids as emulsifiers in food manufacture is not common.

Thanks to the work of Hardy,[1] Harkins,[2] and Langmuir [3] the arrangement of the molecules of emulsifying agents in the liquid/liquid interface is now appreciated. The quantitative basis of the question of arrangement or orientation of the molecules is particularly due to Harkins and his school.[4] Briefly, the essential notion regarding emulsifying agents in general is that they are orientated at the dineric interface, and orientated in such a way as to reduce the free surface energy to a minimum. The polar or active end of the emulsifier is orientated towards the more polar or active liquid, and the less polar end towards the less polar liquid.

The solubility of one liquid in another can be considered as the extreme case of continuously increasing the interfacial separating surface between the liquids, and complete miscibility implies zero interfacial tension. When two non-miscible liquids are in contact, an emulsifying agent will be adsorbed at their interface, if the agent possesses molecular groups which will orientate some to one liquid and some to the other. The emulsifier must, in a sense, act as a bridge for the two dissimilar liquids. By being adsorbed at the interface, the tension there is lessened, and diminished interfacial tension permits increased subdivision of one of the liquids in the other.

THE INVERSION OF EMULSIONS

The globules in an emulsion carry on electric charge due to the adsorption of ions. In the emulsions with which the present volume deals the oil globules are negatively charged. Addition of appropriate electrolytes can lead to this charge being neutralised and the emulsion "breaks," oil separating out.

Where sodium soaps are the emulsifying agents addition of calcium or barium chloride in sufficient quantity will (provided stirring is in progress) not only "break" the O/W emulsion, but lead to the opposite, *i.e.*, W/O type of emulsion. Such a phenomenon is termed inversion, and that point when inversion occurs is the critical point. Obviously, this means that there is a critical ratio

[1] *J. Chem. Soc.*, **127,** 1207 (1925).
[2] *J. Am. Chem. Soc.*, **39,** 354, 541 (1917).
[3] *Chem. Met. Eng.*, **15,** 468 (1916); *J. Am. Chem. Soc.*, **39,** 1848 (1917).
[4] *Am. Coll. Symp. Monog.*, **2,** 141 (1922); Alexander's "Colloid Chemistry" New York, 1926), Vol. I., p. 192.

of the concentrations of Na to Ca or Ba. Much work has been done in this connection by Clowes,[1] Bhatnagar,[2] Seifriz [3] and others.

Sodium soaps promote O/W emulsification, calcium salts promote W/O emulsification. These emulsifying agents are therefore "antagonistic." The multiplicity of ingredients in certain edible emulsions requires the technical chemist to inquire for incompatibles, *i.e.*, for reagents antagonistic to the emulsion type desired. For example, the present writer has a copy of the recipe for making cod-liver oil emulsion as manufactured by a well-known pharmaceutical house. Irish moss and gum acacia are used to disperse the cod-liver oil, but *lime* water is added to the aqueous phase.

In general, as Clowes' investigations showed, electrolytes can be classed into two main groups : (*a*) promoting O/W emulsions—salts of the monovalent cations, Li, Na, K, etc., alkalies, salts of di- and tri-valet anions ; (*b*) promoting dispersion to W/O emulsions—salts of the di- and tri-valent cations Ca, Sr, Ba, Fe, Al.

An interesting case of antagonistic emulsifiers is shown when an attempt is made to emulsify a cold vegetable oil, *e.g.*, olive oil in (say) aqueous gelatin solution. The gelatin favours emulsification to O/W systems. The chilled oil, however, will contain suspended stearin, a finely-divided suspension that is, of a solid more readily wetted by oil than by water. Oil stearin is an excellent emulsifying agent for dispersing water in oil.[4]

The importance of egg-yolk as an emulsifying agent,[5] the use of lecithin [6] in emulsions and chocolate,[7] and the physical chemistry of body fat deposition have drawn considerable attention to the antagonistic emulsifying action of lecithin and cholesterol. Lecithin promotes the formation of oil-in-water emulsions and cholesterol promotes the formation of water-in-oil emulsions. Corran and Lewis [8] studied the effect of lecithin, cholesterol, and mixtures of the two on the interfacial tension between olive oil and water. Despite the fact that the emulsifying agents are of the opposite types, the lowering effect on interfacial tension is additive in the case of their mixtures. With both present in the aqueous phase, inversion of the emulsion occurs when the ratio of cholesterol to lecithin is 1 : 8. If,

[1] *J. Phys. Chem.*, **20,** 407 (1916).
[2] *J. Chem. Soc.*, **117,** 549 (1920) ; **119,** 61, 1760 (1921).
[3] *J. Phys. Chem.*, **29,** 587, 738 (1925) ; *Am. J. Physiol.*, **66,** 124 (1923).
[4] Ayres, U.S. Pat., 1,467,081 (1923).
[5] Wilson, *J. Am. Leather Chem. Assoc.*, **22,** 559 (1927) ; Merrill, *Ind. Eng. Chem.*, **20,** 654 (1928) ; Hanschmidt, *Biochem. Z.*, **59,** 281 (1915) ; Guggenheim, *Z. Immunitäts.*, **61,** 361 (1929).
[6] Haupt, *Deut. Nahrungsmittel-Rundsch.*, (1930), 17 ; Cassanova, *Arch. chim. sci. ind.*, **31,** 282 (1916) ; Magat, *Z. ges. exptl. Med.*, **47,** 211 (1925).
[7] Working, U.S. Pat., 1,781,672 (1930).
[8] *Biochem. J.*, **18,** 1364 (1924). See also Leathes, *Lancet* (1925), 803, 853, 957, 1019 ; Eicholtz, *Biochem. Z.*, **151,** 187 (1924) ; Rona and Deutsch, *ibid.*, **171,** 89 (1926) ; Spranger, *ibid.*, **208,** 164 (1929) ; Califano, *Z. Krebsforschung.*, **26,** 183 (1928) ; Roffo, *Neoplasmes*, **5,** 332 (1926).

however one commences with cholesterol in the oil phase and lecithin in the aqueous phase, the inversion ratio is between 1 : 1 and 2 : 1.

A phenomenon akin to antagonism of emulsifying agents is the creaming of an emulsion by the addition of a new colloid, which itself would promote the *same* type of emulsion. Henry [1] employs starch to ' break ' emulsions, whilst McGavack [2] caused latex to cream by adding pectin, Irish moss or Karaya gum. The phenomenon has been noted by Palmer and Anderson [3] who found albumin to promote creaming of milk, whilst casein retards it. The creaming of milk under the influence of added colloids has been extensively studied by Sirks and van Dam,[4] who found that creaming was due to the adsorption of the new colloid around the fat globules, so leading to clustering (see p. 221).

THE PREPARATION OF EMULSIONS

Emulsification is the art or practice of dispersing a given liquid as more or less permanent globules in another liquid medium. Emulsification is very important industrially, and numerous machines have been devised with a view to securing an intimate subdivision of the disperse phase in the continuous medium. The general principle involved is agitation of the two liquids in vessels which are usually double-jacketed so as to permit of temperature regulation by circulating steam, or hot or cold water as desired. The emulsions are prepared in batches, *i.e.*, intermittently, although continuous-flow emulsifying apparatus are also in use.

Academically considered, the ideal method of preparing emulsions is that wherein the internal phase is projected in a finely-divided condition inside the main bulk of the external phase, *i.e.*, out of contact with a gas phase. The whole work of dispersion can thus be made on the internal phase and adsorption at the dineric interface can be reached without the interfering adsorption at the gas/liquid boundary common to the usual agitation or stirring methods. Conversely mixing liquids by means of blowing gas, *e.g.*, compressed air, through them, is a faulty and unscientific method, since adsorption is pronounced in the resulting froth and not in the liquid/liquid interface. Indeed, good emulsions may actually be " broken " by agitation alone, the protective colloid or emulsifying agent leaving the oil/water interface by preferential adsorption at the air/water interface. Spontaneous cream separation has been reported [5] due to transport influence, and Perks [6] has

[1] Brit. Pat., 322,798 (1928).
[2] U.S. Pat., 1,647,805 (1928) ; Traube, Brit. Pat., 226,440 (1924) ; Johnson and Johnson, Brit. Pat., 286,527 (1927).
[3] Minn. Agr. Expt. Sta., *Ann. Rept.*, (1922), p. 39.
[4] *Verslag. Land. Onderzoek. Rijkslandbouwproefsta.*, **26,** 106 (1922) ; **29,** 137, (1924) ; cf. Green and Halvorson, *J. Infectious Diseases*, **35,** 5 (1924).
[5] Astruc, Aubouy and Dunnan, *Ann. Fals.*, **7,** 418 (1914).
[6] Brit. Pat., 322,416 (1929).

been granted a patent relating to the churning of cream which, in a thin layer, is subjected to continuous vibration of suitable amplitude and frequency.

What we may term the mechanics of emulsification is far from being understood even now in spite of the technical prominence of emulsions.

The effect of rise in temperature is, in general, to make emulsification easier. Reduction of viscosity is obviously a factor concerned. The main interest, however, lies in the relation of temperature to the interfacial tension and the adsorption of the emulsifier at the interface. For non-miscible liquids rise in temperature is accompanied by a decrease in the interfacial tension, a condition favourable to emulsification. With an emulsifying agent soluble in one liquid, change of solubility with temperature may introduce complications. Unfortunately, so little data is available in this field that any emulsion problem must be dealt with as a special case. Where egg-albumin or egg-yolk is used as emulsifying agent the temperature factor is, of course, of first importance, as the heat-coagulated forms undoubtedly differ in emulsifying efficiency from the raw colloids.

The prevalent idea regarding emulsification is that the oil and aqueous phase should be agitated together as violently as possible, an empirical limit only being set on the degree of mixing and the time involved. There is urgently needed a thorough investigation of the effects of different methods of agitation, and the degree of any one kind, on mixing an oil with a standard aqueous phase. Choosing conditions, one can both make and " break " emulsions by agitation. No emulsion study is complete without a size-frequency analysis of the dispersed phase, and such analysis is certainly to be correlated with investigations as proposed.

It is quite reasonable to believe that for any given emulsion apparatus or machine there exists an optimum speed or degree of agitation or mixing, and an optimum time of running, whereby the most stable emulsion can be attained for a given system. Experiments prove this to be correct. Bechold, Dede and Reiner [1] found that the formation of emulsions of water and organic liquids plus finely-divided solids, reached an optimum after ten minutes' standard shaking.

Herschel [2] has investigated the effect of duration of stirring and of speed of agitation when emulsifying lubricating oils in water. He adopted five minutes as the time for agitation (using an electrically-driven paddle) in all his tests, having found that no marked increase in stability of the resultant emulsions followed longer agitation. In regard to the speed of stirring his mixtures of five different oils and water, he found that, in general, there is a speed above which the

[1] *Kolloid-Z.*, **28,** 7 (1921).
[2] U.S. Bur. Standards, *Technolog. Papers*, No. **86,** (1917).

stability of the emulsions decreased, *i.e.*, the curve representing the relation between speed and stability passes through a minimum. Fig. 19 is reproduced from Herschel's paper ; the abscissæ represent speed in hundreds of revolutions per minute. whilst the ordinates measure stability in terms of the number of c.c. of clear oil rising to the top of the emulsion per hour. He adopted a speed of 1,500 r.p.m. as giving, on the average, a minimum rate of settling.

Under constant conditions of agitation the graph connecting

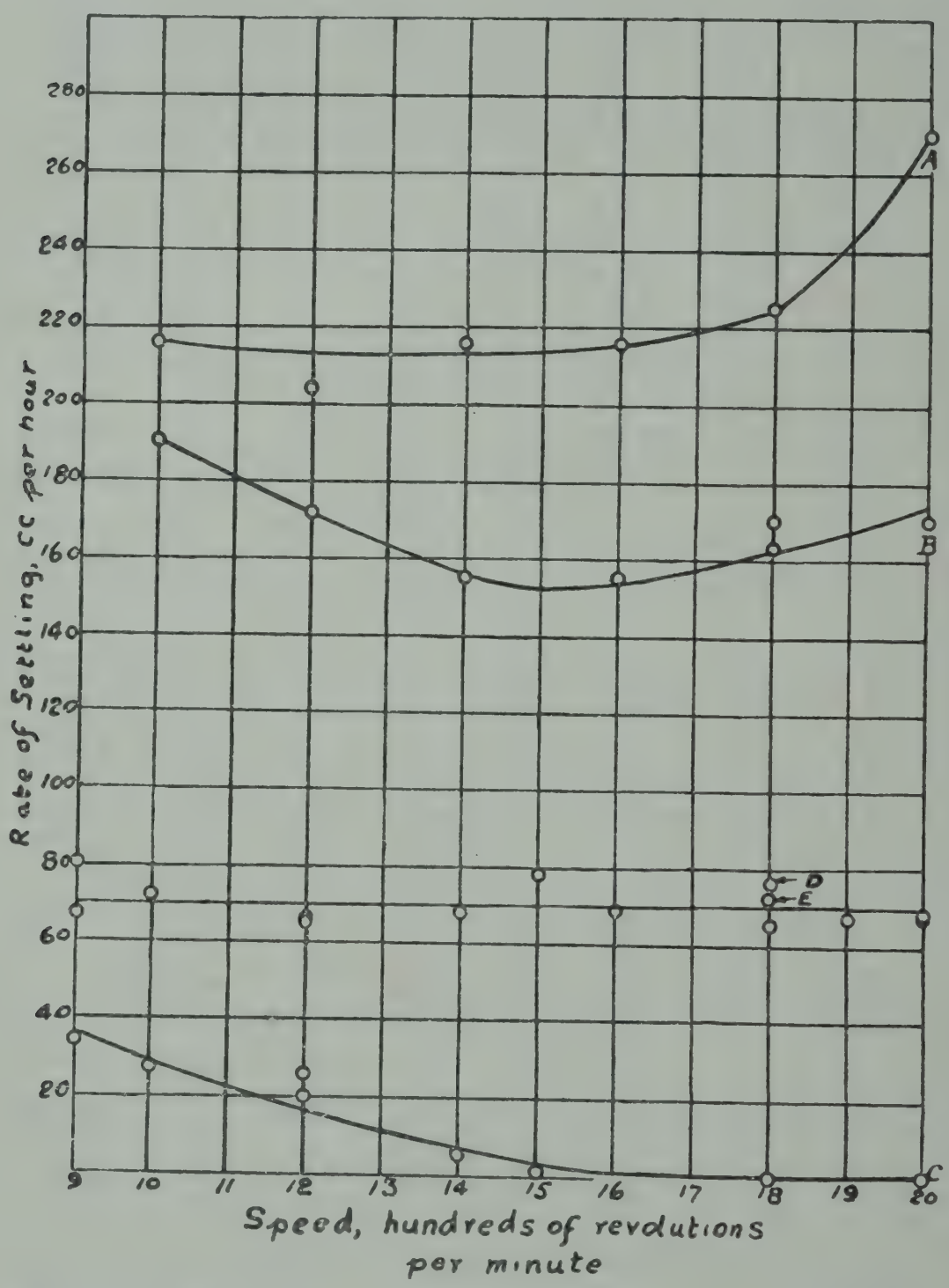


FIG. 19.—Effect of speed of paddle upon rate of settling (stability) of emulsions.

time with emulsification effected is of the exponential type. Fig. 20 is based on experimental data by Briggs and Schmidt.[1] The practical significance of such results is obvious, inasmuch as excessive expenditure of power in making emulsions is unnecessary, an optimum working time being readily ascertained.

Interesting work on emulsification has been carried out by Briggs,[2] who, arguing that the *mode* of shaking a mixture of benzene

[1] *J. Phys. Chem.*, **19**, 478 (1915).
[2] *Ibid.*, **24**, 120 (1920).

and sodium oleate might be an important factor, found that *intermittent* shaking was much more effective than the usual uninterrupted shaking. Briggs concluded that " intermittent shaking may be six hundred or even a thousand times more effective than uninterrupted but equally violent agitation."

Shaking by hand, mixtures of benzene in 1 per cent. aqueous sodium oleate, Briggs found that to emulsify 60 per cent. by volume of benzene 750 shakes were necessary, occupying 4·2 minutes. The same mixture could be completely emulsified with 5 shakes in less than 1 minute, if after 2 shakes a rest of 30 seconds was permitted. It was observed that the time required to form a complete emulsion with intermittent agitation passes through a minimum as the rest interval increases. There is general agreement of the value of intermittent agitation in preparing a stiff emulsion such as mayonnaise.

Woodman [1] has investigated the problem and suggests that the efficiency of intermittent emulsification is due to the increased facilities for adsorption offered by the rest interval. His ideas deserve testing by a study of the degree of adsorption at a liquid/liquid boundary in relation to time. The present writer believes that where the dispersed phase is injected into the continuous medium, below the surface, intermittent injection would confer no advantage.

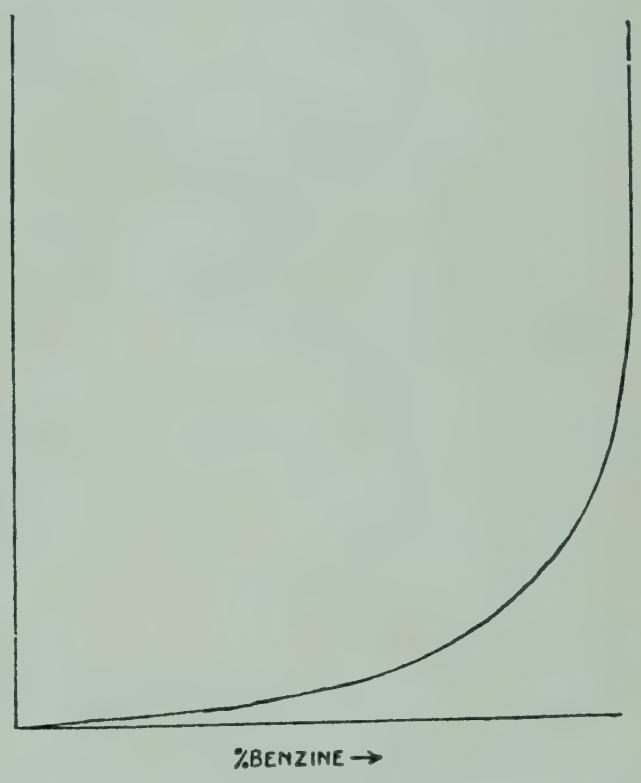


FIG. 20.—Emulsification in relation to time.

BUTTER

Butter is the fatty food resulting from churning cream and working the separated butterfat granules into a plastic mass. An approximate analysis [2] of butter may be taken as : fat = 82·3 ; water = 13·9 ; salt 2·6 ; curd = 1·1 per cent.

Cream is agitated in a churn, thickening thereby until it has a pronounced rigidity. The fat aggregates grow in size and enmesh the milk serum. At the same time the air incorporated by the agitation produces a foam which assists the resistance of the churn contents to agitation. A point is suddenly reached in churning when the fat agglomerates have reached dimensions when further entrainment of liquid ceases and the butter "comes," whey recedes

[1] *J. Pomology Hort. Sci.*, **4**, 95 (1925).
[2] U.S. Dept. Agr., Bur. An. Ind., *Bull.*, **149**.

off the butter clumps, and many butter granules become visible to the naked eye. Churning occupies 40 to 60 minutes, but the butter "breaks" or "comes" with amazing suddenness. The butter granules are afterwards worked or pressed to a homogeneous fatty mass.[1]

The physical conditions of churning, especially the temperature, exert a profound influence on the butter, particularly with regard to its moisture content. The main factors to be considered in this connection are the fat content, the acidity, and the viscosity of the cream, and the agitation employed. Jørgensen [2] points out that other factors influencing the yield are the size of the fat globules and the extent of their clumping together in cream. Cream with large fat globules churns the easiest,[3] an observation confirmed by Van Slyke,[4] whose study showed that milk from breeds giving the larger globules, churned the more readily, and that churning was more prolonged as the lactation period increased.

A cream containing 30—45 per cent. of fat churned at a temperature ranging from 13°—18° C. should give good results. As cream ripens, lactic acid is produced, the viscosity of the cream diminishes and churning becomes easier. The casein is coagulated and prccipitated if the acidity is too pronounced, and casein clots may be found in the butter mass. Hittcher [5] found that the initial churning temperature of the cream and its fat content have hardly any influence on the size of the agglomerates of butter formed. He made the interesting observation that freezing the cream prior to churning at usual temperatures greatly accelerated churning. Probably this is due to some change in the physical characteristics of the protein envelope.

Under the microscope butter is seen to contain numerous water globules, and Boysen,[6] in his study of the structure of butter as investigated by photomicrography through both parallel and crossed Nicol prisms, gives the following data for the distribution of the water globules.

TABLE 30. WATER GLOBULES IN BUTTER.

Average Diameter.	Number of Globules.
1·9μ	12,905,700 per c.c.
4·0	230,490 ,,
7·5	61,469 ,,
12·5	10,740 ,,
20·0	628 ,,

[1] An excellent detailed account of the technology of churning is given by Hunziker, "The Butter Industry" (Illinois, 1927), Chap. 14.

[2] *Molkerei Ztg.*, **40,** 1772 (1926).

[3] Babcock, 4th Intern. Rept. N.Y. (Geneva) Agr. Expt. Sta. (1885), p. 297.

[4] *Ibid.*, 10th Intern. Rept. (1891), p. 385.

[5] *Landw. Jahrb. Schweiz.*, **51,** 489 (1918).

[6] *Milchwirtschaft. Forsch.*, **4,** 1 (1927).

TABLE 30.—WATER GLOBULES IN BUTTER—*contd.*

Average Diameter.	Number of Globules.
30·0	139 per c.c.
40·0	57 ,,
50·0	25 ,,
60·0	12 ,,
70·0	8 ,,
80·0	5 ,,
90·0	4 ,,

According to Storch,[1] a cubic millimetre of butter contains from 2—13 million water globules, having diameters less than 10μ, and from 4—6 thousand water globules, with diameters over 10μ. Rahn and Boysen [2] find that ordinarily butter contains 12—18 billion water globules per gram; the majority, amounting to approximately 7 per cent. of the moisture, are smaller than 15μ. About 2 per cent. of the moisture is present as globules of diameters 15 to 100μ, whilst the remaining 4 per cent. of moisture is present as globules larger than 100μ.

There are fat globules also in butter, and King [3] has published his analysis of the size-frequency of the fat-globules following his examination of 10 samples. Thus, the average grading of each 100 globules shows :—

Average Diameter.	Number of Globules.
2·6μ	8·45
3·9	49·55
5·2	33·80
6·5	6·95
7·8	0·85
9·1	0·275
10·4	0·125

Butter also contains entrained air, as will be discussed later (see p. 182). It is now agreed [4] that butter is a solid system with a fatty continuous phase in which are dispersed fat globules, water globules and air bubbles, all enveloped by hydrated protein. In all probability, the fatty continuous medium is the more liquid fraction of butterfat, pressed out from the fat globules during churning and working. The general emulsion type is that of water-in-oil.

The fundamental initial problem presented by butter is concerned with the churning of cream, an oil-in-water emulsion, into butter, an emulsion of the opposite type. Modern investigations

[1] *Beretning frä den Kgl. Vet. og Landbohöjskole Lab.*, **36,** 1 (1897). See also Palmer, *Am. Coll. Symp. Monog.*, **1,** 410 (1923).

[2] *J. Bact.*, **15,** 30 (1928).

[3] *Milchwirtschaft. Forsch.*, **8,** 98 (1929).

[4] King, *ibid.*, **8,** 423 (1929); Holm, "Fundamentals of Dairy Science" (New York, 1928), p. 182.

have cleared up the matter, which is not so simple as a recent writer [1] would have it : " The churning of cream to butter is the outstanding example of breaking an emulsion by mechanical action. The churning is supposed to split open the layer of emulsifying agent (casein) so that the droplets of the oil phase are able to coalesce."

As to the exact changes occurring when milk-fat is churned into butter, there is still divergence of opinions. The supporters of the " slim-membran " theory argued that the mucoid substance enveloping the fat globules is rubbed off and the globules thereupon coalesce.[2] Fleischmann [3] inclines to regard the process of churning as being the solidification of superfused fat globules, but this theory is discredited by the fact that the fat globules in milk are rapidly solidified by mere cooling.[4]

From the modern schools two outstanding theories have been proposed : (1) the Phase-Inversion Theory ; (2) the Foam Theory.

The Phase-Inversion Theory. The Fischer-Hooker Theory of emulsions (see p. 169) has been applied to milk and butter.[5] Milk is considered as an emulsion of butterfat in hydrated protein. Full cream milk shows little tendency to form butter, nor even does cream. According to Fischer and Hooker the tendency of cream to form butter is assisted by the dehydration of the protein with which the water is so combined, *e.g.*, by souring the cream. They write : " Since the hydrated colloids tend to collect in the surface layers between the fat particles and the aqueous phase of the cream, efforts are made to break these layers, and so to hasten coalescence of the fat droplets, by churning. *The combined efforts therefore bring about a progressive increase in the concentration of the oil with a decrease in the concentration of the hydrated colloid until the instability of the oil-in-hydrated colloid emulsion becomes so great as to ' break ' and yield the hydrated colloid-in-fat emulsion which we call butter.*"

Palmer [6] supports this theory, studying the inversion of emulsion type by staining the fat globules in cream with oil-soluble dyes before churning. He [7] also demonstrated by electrical conductivity measurements [8] that the resistance of cream at constant temperature during churning increases to a maximum, remains stationary for about 30 minutes, and then drops rapidly to the original value. The point of maximum resistance is identified with the completion of the gradual inversion of the phases. The " breaking " of the butter and the release of the buttermilk at once restore the electrical conductance.

[1] Davey, *Am. Coll. Symp. Monog.*, **4,** 41 (1926).
[2] Storch, *Analyst*, **22,** 197 (1897) ; Beau, *Rev. gén. Lait.*, **2,** 15 (1903) ; Richmond, *Analyst*, **29,** 185 (1904).
[3] *J. Landw.*, **50,** 33 (1902).
[4] For a recent revival of the theory see Pratolongo, *Giorn. chim. ind. applicata.*, **6,** 3 (1924).
[5] " Fats and Fatty Degeneration " (New York, 1917), p. 93.
[6] Missouri Agr. Expt. Sta., *Bull.*, **163,** 40 (1919).
[7] *Am. Coll. Symp. Monog.*, **1,** 411 (1923).
[8] *Vide* Clayton, *Brit. Assoc. Coll. Repts.*, **2,** 114 (1918).

The present writer would point out that true inversion of emulsions is not indicated. There is a release of the fat globules from their protein environment, and this release would result in a complete fat separation did the fluidity of the fatty phase permit. (Thus, North [1] agitates cream until the fat globules agglomerate and then treats with hot water so as to separate the butterfat as an oily layer). One cannot consider churned cream with its butter granules as an emulsion at all ; it is in fact a suspension of loose fatty granules within which the aqueous medium is also enmeshed. By purely mechanical working these granules are pressed together so that the homogeneous butter mass contains aqueous globules because of incomplete expression of buttermilk. The resemblance of butter to a water-in-oil emulsion is thus accidental. What really demands explanation is why milk or cream emulsions lose stability or *break* (not invert) upon being agitated, and the Foam Theory seeks to provide such explanation.

The Foam Theory. As already stated, the violent churning of cream causes the incorporation of air and the formation of a foam. Then quite suddenly the foam collapses and almost simultaneously the butter nuclei grow to macroscopic size. Siedel showed that in churned cream prior to the foam collapsing, nearly all the fat had accumulated in the foam and the liquid menstrum had become very poor in fat. Also, he proved that cream from churned milk which no longer frothed, would not yield butter. On the basis of these observations and a study of skim milk froth, Rahn [2] proposed the following theory of butter formation.

In the fat-rich foam of churned cream there is a close packing of the fat globules in the froth lamellæ, finally leading to fat clumps. The protein in the froth lamellæ gradually assumes a solid character (irreversible) and continued churning destroys the structure, whereupon the foam collapses. Further working brings about the gradual union of the fat globules, and simultaneously with the collapse of the foam the fatty agglomerates are large enough to become visible ; the two phenomena have a common origin.[3]

Practical experience supports this theory. Butter can be made even at temperatures where the fat is quite liquid, and Rahn's theory shows no necessity for the fat to be solid. Foam formation and " precipitation " of the foam-colloid occur at higher temperatures. The highest limit for butter formation (34° C.) is fixed by the mechanical breaking down of the fat globules and the newly-formed butter nuclei. This rapidly increases with rising temperature, so that at 50° C. after churning new milk for 1 hour, 83—87 per cent. of the fat, and after 2 hours' churning 100 per cent. of the fat, is completely

[1] U.S. Pat., 1,416,053 (1922) ; Brit. Pat., 206,918 (1922).

[2] *Kolloid-Z.*, **30,** 341 (1922). *Vide* " Physik der Milchwirtschaft," (Berlin. 1928), p. 81 *seq.*

[3] See also Mohr and Brockmann, *Milchwirtschaft. Forsch.*, **10,** 173 (1930).

homogenised and will not separate from the skim milk when centrifuged.[1]

Rahn [2] has shown that the inability to churn cream at low temperatures is due to the non-cohesion of the solid fat globules, although even at 2° C. there is distinct evidence of a clumping tendency. He mentions [3] too, that *a full churn with no air space will not yield butter* even after 2 hours' agitation. Rahn's theory receives additional support from experiments by Pedersen,[4] who washed cream with water prior to centrifuging and then subjected the cream to bacterial action to effect changes in the protein content. Such cream would not churn into butter. Pedersen also made an emulsion of butterfat in skim-milk, dispersed to a degree comparable with fresh milk. Churning was very difficult to accomplish and butter formation was incomplete. The resulting butter resembled margarine.

Finally, Rahn showed that a thin layer of butter left under the microscope for a few days gives a clear edge due to water evaporation, and in this clear rim can be seen the delicate outline of the envelopes of individual *fat* globules, which according to the treatment of the butter are still more or less spherical. The recent work of King [5] gives strong support to these findings.

Besides churning cream, other methods have been proposed for obtaining butter. Clavel [6] claims that by converting milk into froth by streaming gases through it, the cream fat may be obtained by now pouring through a fine sieve. This is apparently an old art,[7] but it has modern application in other fat-separating processes.[8] A variant of the method is due to Salenius,[9] who boils cream at low temperature *in vacuo :* agitation causes butter separation.

Alexander [10] proposes the heating of milk under pressure so as to dissolve casein and thus facilitate the separation of butter fat. Stevenson [11] suggests the addition of hydrochloric acid to milk until the pH value of the serum is 3·0, the precipitated casein thus being redissolved. The fat is now removed by centrifuging. These are but instances of the fact that the *sine qua non* of butter-making is the removal of the protein adsorbed layer round the butterfat globules.

The Air Content of Butter. The practice of churning cream, followed by the working of the butter, inevitably leads to the incorporation of air. Rahn and Mohr [12] examined 290 samples of

[1] Siegfeld, *Molkerei-Ztg.*, **22,** 287 (1908).
[2] *Forsch. Gev. Milchw.*, **2,** 76 (1922) ; *Kolloid-Z.*, **30,** 341 (1922).
[3] " Physik der Milchwirtschatt," p. 86.
[4] *Molkerei-Ztg.*, (1918), 176.
[5] *Milchwirtschaft. Forsch.*, **8,** 93, 423 (1929).
[6] German Pat., 314,090 (1918).
[7] *Vide* Rahn, " Physik der Milchwirtschaft," p. 86.
[8] Karpinsky, Brit. Pat., 177,498 (1922) ; Edser, Brit. Pat., 157,490 (1922).
[9] Brit. Pat., 333,233 (1929).
[10] U.S. Pat., 1,401,853 (1921).
[11] U.S. Pats., 1,397,663 and 1,397,664 (1921).
[12] *Milchwirtschaft. Forsch.*, **1,** 211 (1924). Cf. Pickerill and Guthrie, Cornell Univ. Agr. Expt. Sta., *Bull.*, **355** (1915).

butter and found the mean gas content to be 4·2 c.c. in 100 gr. butter, the extreme values being 0·97 c.c. and 9·38 c.c. Rogers [1] found the gas content of butter to be 10 per cent. by volume, analysing; oxygen, 20 per cent.; nitrogen, 33 per cent.; and carbon dioxide, 47 per cent. The percentage of incorporated air in butter depends on the degree of working of the butter. Guthrie [2] recently examined this question and obtained the average value, 4·65 per cent. of air, for fairly well worked butter, and 5 37 per cent. for thoroughly worked butter.

Salted Butter. After butter has been worked from the granules into a fatty mass, salt is added, usually as a dry fine-grained powder.[3] The whole is now thoroughly kneaded [4] so as to effect uniform solution and distribution of the salt. Owing to osmotic effects salt causes coalescence of the water globules. If the subsequent blending is not sufficient to distribute the salt and to break up the resulting *brine* globules, mottles occur and the butter colour is patchy.

Boysen [5] has determined the water distribution in salted and unsalted butters. The major portion of the water globules have diameters below 15μ. Thus :—

Globule Size.	Unsalted.		Salted.	
	Number.	Per cent.	Number.	Per cent.
$<3\mu$	17,175,400	6·47	11,066,800	3·97
3—5μ	317,540	1·12	180,700	0·61
5—10μ	72,080	1·67	60,230	1·33
10—15μ	9,040	0·97	11,570	1·18

He gives the following table, which is of much interest :—

TABLE 31. WATER DISTRIBUTION IN FRESH BUTTER AND IN SALTED BUTTERS ONE DAY MADE.

Globule Size μ.	Fresh Butter.	Salted A.	Salted B.	Salted C.	Salted D.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Group 1, <15 . .	11·33	6·95	6·55	6·26	7·31
Group 2, 15—100 .	1·37	1·22	1·41	1·80	1·33
Group 3, >100 . .	1·50	4·23	4·44	4·29	3·66
Water content . .	14·20	12·40	12·40	12·35	12·30

[1] U.S. Dept. Agr. Bur. An. Ind., *Bull.*, **162**, p. 5.
[2] *J. Dairy Sci.*, **13**, 461 (1930).
[3] A detailed account of technical practice is given by Hunziker, "The Butter Industry" (Illinois, 1927), 318—356.
[4] The influence of working on the composition of butter is discussed by Manhart, *J. Dairy Sci.*, **11**, 52 (1928).
[5] Discussed by Rahn, "Physik der Milchwirtschaft," p. 104 *seq.*

Mottled butter (white opaque dapples) results from the localisation of numerous very small water globules. Mottling is only seen in salted butter, which is insufficiently or unevenly worked, *i.e.*, the water globules and the brine globules have not been mixed and evenly redispersed. Boysen [1] gives the following data relative to the size of the aqueous globules in the dark and the lighter portions of butter respectively.

TABLE 32. SIZE OF AQUEOUS GLOBULES IN MOTTLED BUTTER

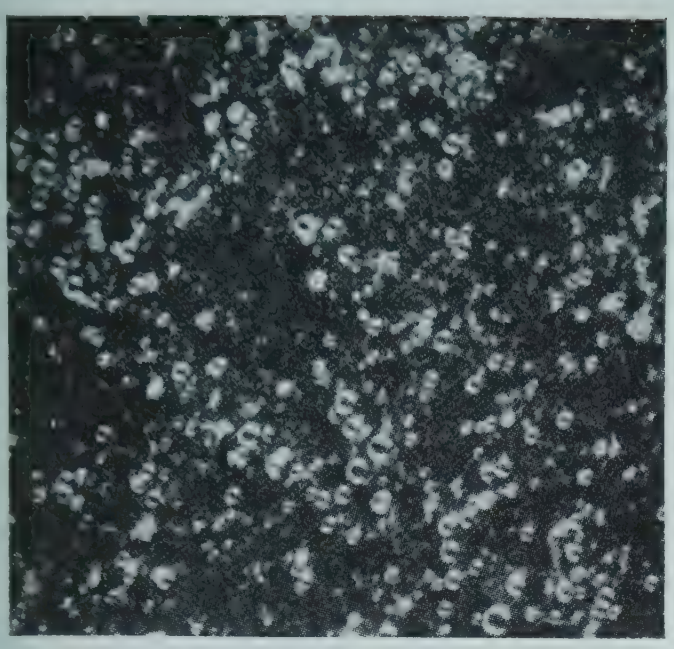
Globule Size.	Mottled Butter.	
	Dark.	Light.
	Per cent.	Per cent.
$<15\mu$	2·89	6·21
$15—100\mu$	0·86	0·56
$>100\mu$	9·09	6·25
Water content . .	12·84	13·02

Hunziker [2] has published excellent photomicrographs of butter illustrating the influence of working on salted as well as on fresh butter, and showing also the globules in mottled butter (Figs. 21 and 22). The mottles are due to " the small size, high curvature and large number " of the very small globules, which " bend, refract and deflect the rays of light to such an extent that the butterfat does not appear in its natural colour "—yellow.

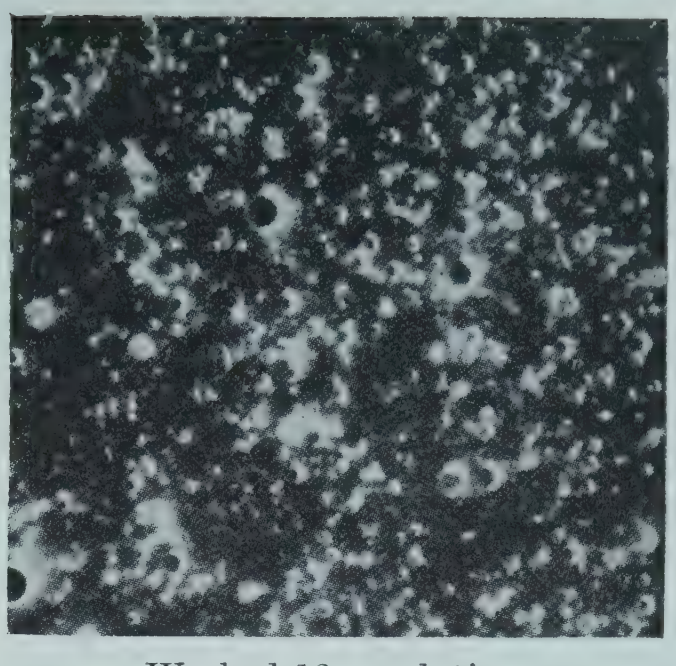
The Water/Fat Interface. Palmer [3] has calculated that the water/fat interface in 1 lb. of butter may equal 1,000 square metres, a factor by no means negligible when considering the question of rancidity.[4] King [5] has taken data by Boysen [6] and calculated the total surface area of the water globules, so obtaining the figures in Table 33 on p. 185.

Whilst Palmer's calculation gives an interfacial area of 2,220 square metres per 1 kilogramme of butter, King finds only 154 square metres, and connects the difference with the greater diameter

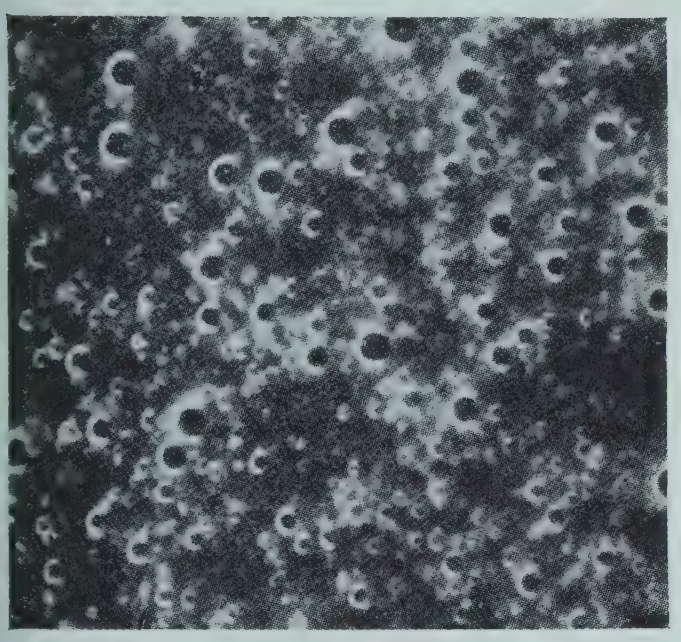
[1] *Milchwirtschaft. Forsch.*, **4,** 221 (1926).
[2] *J. Dairy Sci.*, **3,** 77 (1920); " The Butter Industry," p. 534.
[3] *Ind. Eng. Chem.*, **16,** 634 (1924); *Proc. World's Dairy Congress*, **2,** 1165 (1923).
[4] Cf. Rahn and Boysen, *J. Bact.*, **15,** 30 (1928).
[5] *Milchwirtschaft. Forsch.*, **9,** 501 (1930).
[6] *Ibid.*, **4,** 237 (1927).



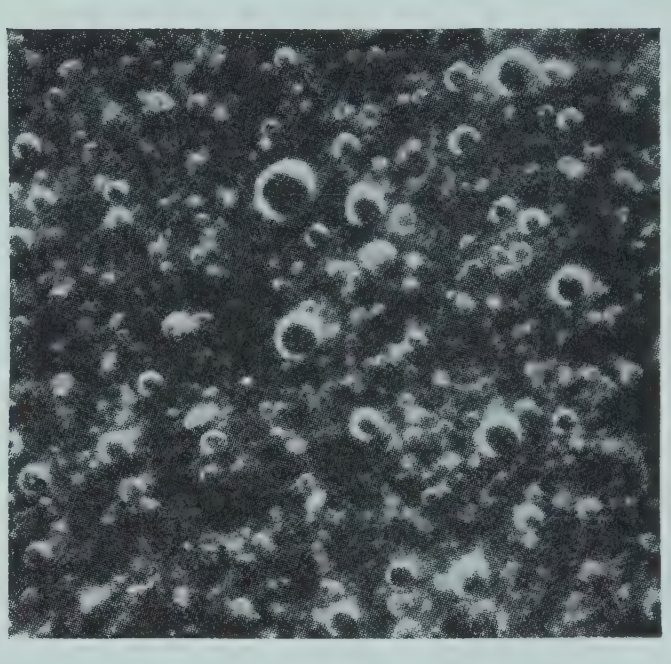
Worked 6 revolutions.
No mottles.



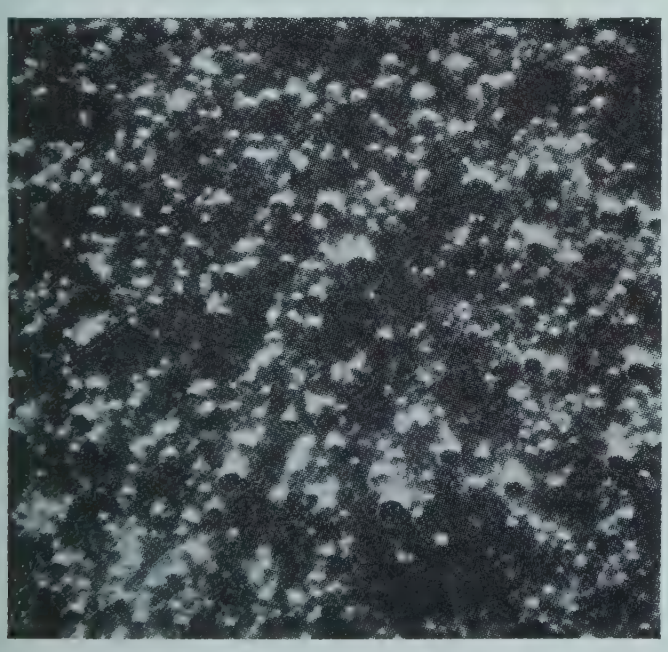
Worked 12 revolutions.
No mottles.



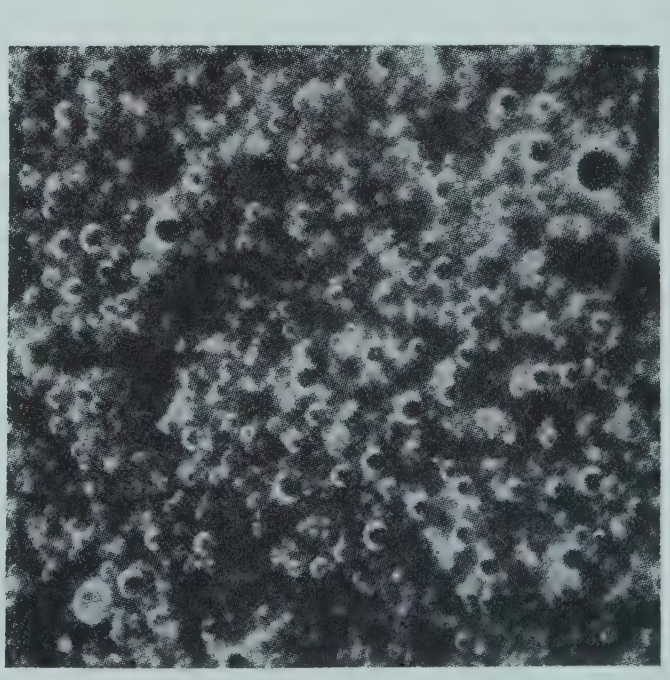
Worked 18 revolutions.
No mottles.



Worked 26 revolutions.
No mottles.



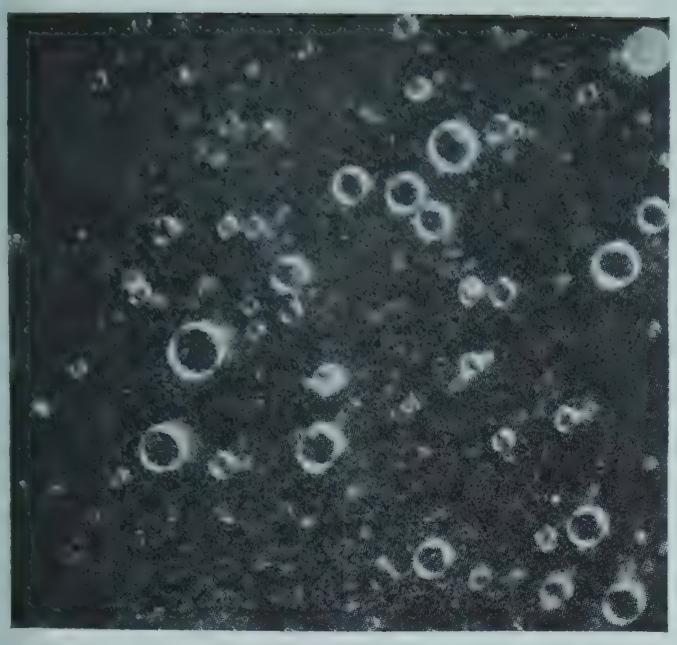
Worked 34 revolutions.
No mottles.



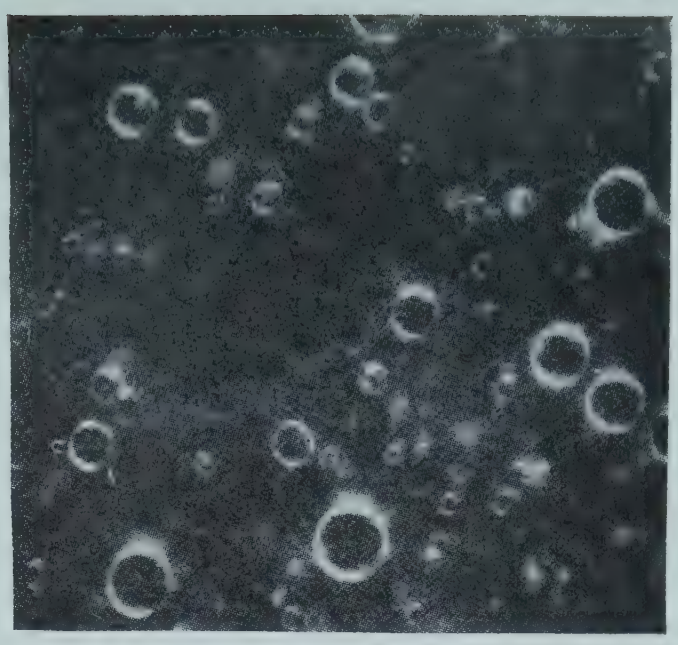
Worked 66 revolutions.
No mottles.

FIG. 21.—Size of water droplets in unsalted butter during working process. (Magnification 740.)

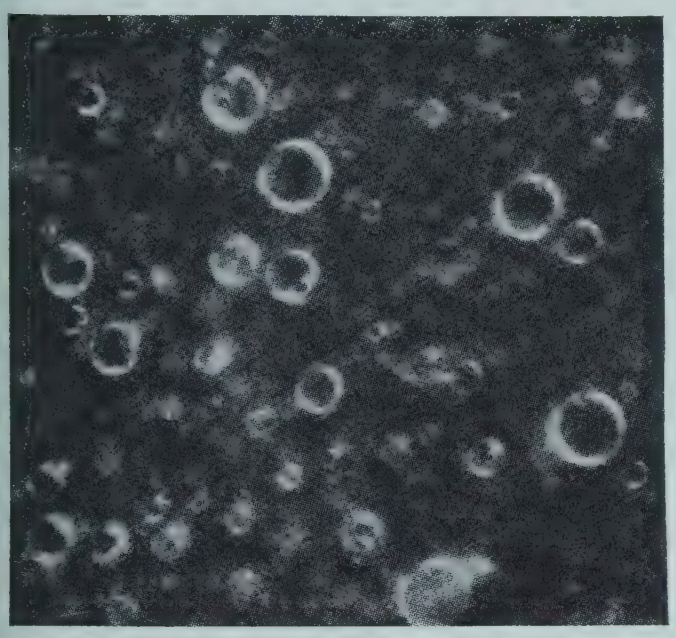
[*To face p.* 184.



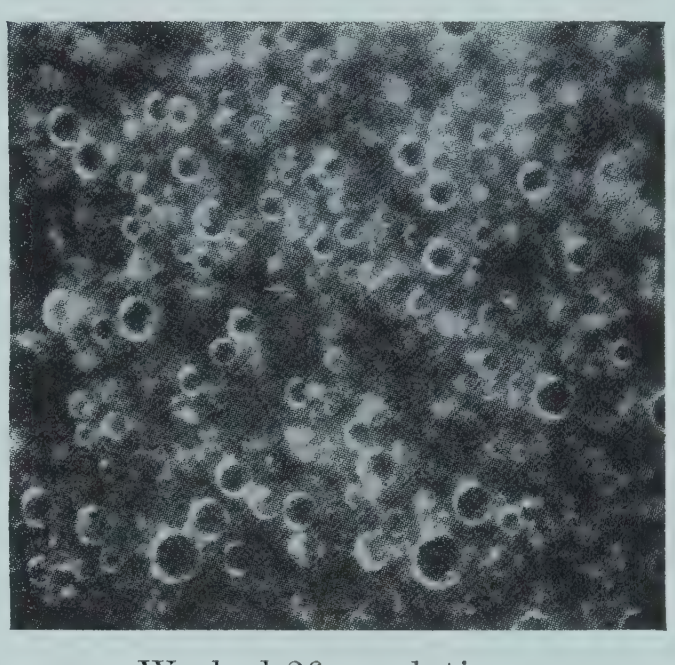
Worked 6 revolutions.
Mottled.



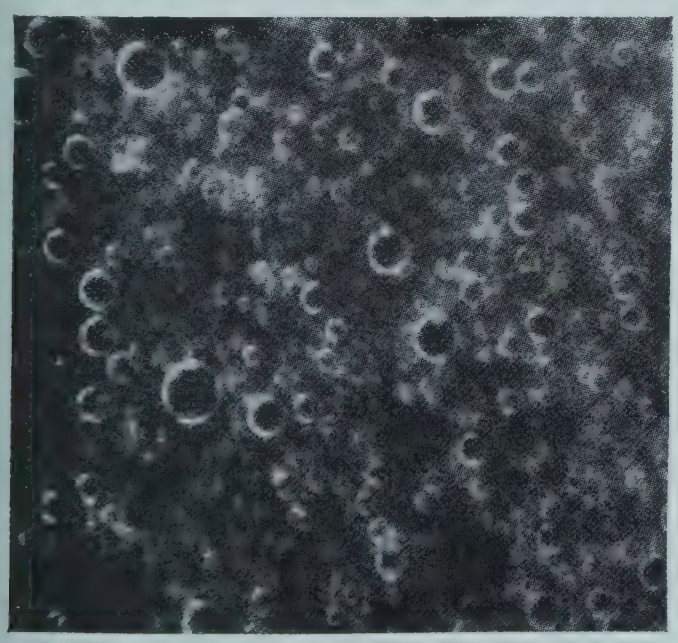
Worked 12 revolutions.
Mottled.



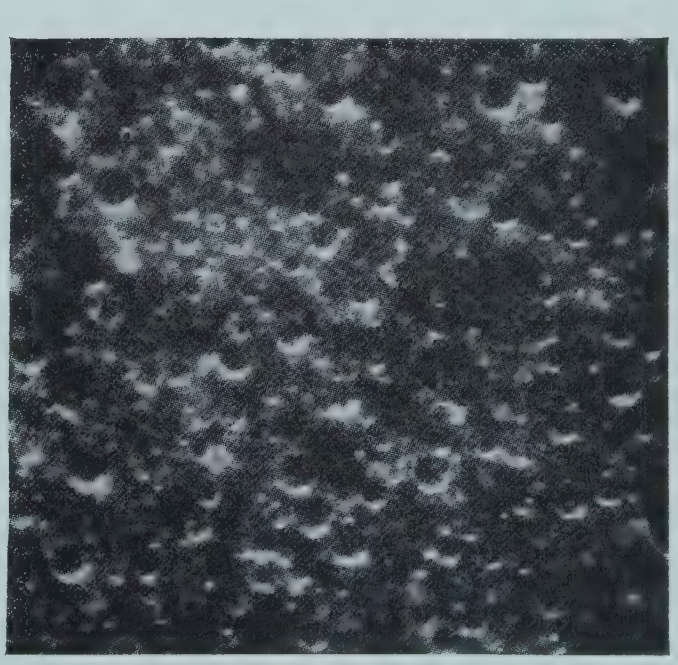
Worked 18 revolutions.
Mottled.



Worked 26 revolutions.
Slightly mottled.



Worked 34 revolutions.
No mottles.



Worked 66 revolutions.
No mottles.

FIG. 22.—Size of water droplets in salted butter during working process.
(Magnification 740.)

[*To face p.* 184.

Table 33. Calculation of the Interface Water/Fat in Butter

Group.	Average Diameter in μ.	Interfacial Area in μ^2.	Fresh Butter.				Salted Butter.			
			Number of Water Globules in 1 cm.^{3}.	Water Content. Per cent.	Interfacial Area in 1 cm.^{3} Butter.		Number of Water Globules in 1 cm.^{3}.	Water Content. Per cent.	Interfacial Area in 1 cm.^{3} Butter.	
(After Boysen).		(Calculated).	(After Boysen).		cm.^{2} (calculated).		(After Boysen).		cm.^{2} (calculated).	
I	1·9	11·3	17,175,400,000	6·47	1,948	2,279	11,066,800,000	3·97	1,255	1509
	4·0	50·3	317,540,000	1·12	160		180,700,000	0·61	91	
	7·5	176·7	72,080,000	1·67	127		60,230,000	1·33	106	
	12·5	490·6	9,040,000	0·97	44		11,570,000	1·18	57	
II	20·0	1256·0	620,000	0·27	8	19	600,000	0·25	8	25
	30·0	2826·0	115,000	0·17	3		160,000	0·23	5	
	40·0	5025·0	46,000	0·16	2		69,000	0·23	4	
	50·0	7850·0	20,000	0·14	2		29,000	0·19	2	
	60·0	11310·0	8,500	0·10	1		16,000	0·18	2	
	70·0	15400·0	6,000	0·11	1		12,000	0·22	2	
	80·0	20100·0	2,500	0·07	1		6,500	0·17	1	
	90·0	25430·0	2,500	0·10	1		5,100	0·19	1	
I	>100·0	—	—	10·28	extrapolated.	6	—	3·61	extrapolated.	10

of Palmer's water globules. *Apropos* of this, King provides Table 34, showing that the major portion of the interface is related to the small globules.

TABLE 34. RELATION OF GLOBULE-GROUPS TO THE TOTAL INTERFACE

Globule-Group (Boysen).	Fresh Butter.	Salted Butter.	Remarks.
	Per cent. of Total Interface.		
I.	98·9	97·7	Buttermilk globules.
II.	0·8	1·6	Wash-water globules.
III.	0·3	0·7	

That a relation does exist between the size and distribution of the water globules in butter is supported by the observations of Mundinger,[1] who showed that rancidity under the influence of soluble iron salts (iron lactate) is related not only to the amount of such salt but to the extent of its distribution *via* the aqueous phase.

King [2] has recently reviewed in some detail the question of the colloid structure of butter and puts forward the view that the system is really one of butterfat and water globules dispersed in a liquid fat fraction. The total butterfat is therefore present in two forms. The continuous phase portion is stated to contain 1·5 times the quantity of volatile fatty acids present in the fat globules.

MARGARINE

Margarine [3] is a substitute for butter, certain animal and vegetable oils and fats replacing butterfat. The oils and fats are liquefied together in certain proportions depending on the quality of the margarine required, and then churned with soured milk to form an emulsion. This emulsion is rapidly cooled, either by means of a spray of ice-cold water under pressure, or by flowing on to well-cooled revolving drums. The product in each case is then worked up by suitable kneading machinery to the required butter consistency and texture.

[1] *Ibid.*, **7**, 327 (1929).
[2] *Ibid.*, **9**, 501 (1930).
[3] *Vide* Clayton, "Margarine" (London, 1920).

A typical margarine has a fatty composition as follows :—

	Per cent.
Premier jus	15
Coconut oil	15
Palm-kernel oil	50
Ground-nut oil	10
Cotton-seed oil	10
	100

The mixture of oils and fats is melted and then intimately mixed with milk, which has previously been " ripened " or " soured " by inoculation with certain strains of lactic acid micro-organisms. In the case of margarine intended for bakers' use, milk is replaced by water.

The most essential factor in the production of a margarine of a butter-like texture, is the preparation of a stable and *suitably-phased* emulsion,[1] containing from 77 per cent. to 84 per cent. fat and 23 per cent. to 16 per cent. water (milk serum). The milk used is at a temperature of about 7° C., and the melted mixture of oils and fats at about 40° C. Before actually chilling the emulsion its temperature is reduced to about 24° C., so that a thick custard-like emulsion ensues, of greatly enhanced stability. This greater stability is due to the viscosity of the system, and furnishes an instance where viscosity is desirable in emulsion preparation.[2] The object of chilling the emulsion is to fix the fine-grained condition arrived at, whereby the desirable intimate and finely-divided mixture is maintained in the solid state. Any " breaking " of the emulsion would lead to the formation of unemulsified fat, whereby the texture of the final product would be unsatisfactory.

Two types of emulsifying machines are in common use in margarine manufacture. The first is essentially a jacketed tank, permitting temperature control by circulating either hot or cold water. Milk is admitted to the tank, and then the oil mixture slowly run in, whilst constant agitation is maintained, *e.g.*, by means of rapidly-revolving baffle plates.

It is absolutely essential to run the oils into the milk, and not *vice versa*. Thus a fine-grained emulsion results, of the oil-in-water type, which on cooling to 24° C. (with constant agitation) becomes remarkably permanent. The more slowly the oil is run in the better the results obtained.[3]

If, on the other hand, the oils be in the churn in bulk and the milk run in, an emulsion of the water-in-oil type ensues. The same

[1] Clayton, *Margarine Journal*, **2**, 503 (1920).
[2] Hillyer, *J. Am. Chem. Soc.*, **25**, 513 (1903).
[3] *Vide* Eichstädt, *Milchwirtschaft. Forsch.*, **9**, 388 (1930).

thing happens if both phases are placed in bulk in the churn (say 80 per cent. oil and 20 per cent. milk) without undue mixing, and then the agitating device set in motion. On consideration of volume factors alone, it will be evident that if one has the oil phase in bulk and milk is introduced, stirring continually, the conditions from the purely mechanical aspect of disintegration of particles favour the formation of the water-in-oil type of emulsion. Such an emulsion is not "permanent." As soon as the agitation ceases, separation into layers occurs, owing to the fact that the very nature of the emulsifying agents present, viz., milk colloids, demands the oppositely-phased system. Of course, this water-in-oil type emulsion can be "fixed" by any of the methods of rapid chilling used in margarine manufacture, but even then, the solid emulsion produced is far from satisfactory in the subsequent processes, and may well lead to a final margarine of poor texture.

The second type of emulsifying machine aims at the continuous production of emulsions, whereby there is economy in space, time, and power. The principle underlying continuous churns is the simultaneous admission of the oils and milk into a thermo-regulated chamber, whence, after some suitable device has ensured intimate mixing, an emulsion is discharged in a continuous stream.[1] It is at once apparent that the same degree of freedom of the control of physical or mechanical factors is not permitted with this churn as with the older tank type. In the writer's experience, even when using milk and oils, *i.e.*, when the purely physico-chemical conditions are satisfied, different mechanical treatments may lead to either the oil-in-water or water-in-oil type of emulsion. Sometimes the passage through a continuous churn (wherein blades revolving 1,600 r.p.m. cause violent agitation) of a margarine emulsion of the oil-in-water type, will completely reverse the type of emulsion. Probably volume factors exercise a marked influence in such inversions.

It may be noted here that the nature of an emulsion may be determined in several ways. Staining with an oil-soluble dye such as Sudan III. or Oil Red O Pyridin [2] will show on examination with the microscope whether the fatty phase is the internal or the external medium. This method is particularly good with solidified emulsions, such as butter or margarine. An electrical method was proposed by the writer,[3] based on the idea that although a small electrical conductance may be observed in the case of an emulsion of the O/W type, practically no conductance would be observed in the case of a W/O type, since the continuous medium is a fatty

[1] Well-known types of continuous churns : Schroder, Brit. Pat., 25,404 (1905); German Pat., 309,717 (1917); Risberg, Brit. Pat., 25,890 (1907); Silkeborg, Brit. Pat. 4,657 (1914); Biichfeldt, Brit. Pat., 18,048 (1914); Leitch, U.S. Pat., 1,266,501 (1918).

[2] Proescher, *Stain Tech.*, **2**, 60 (1927).

[3] *Brit. Assoc. Coll. Repts.*, **2**, 114 (1918).

phase, *i.e.*, an electrical insulator. The method works well, using electrodes 1 inch apart, with an E.M.F. of about 110 volts.

Recently, Carrière [1] has devised a method of determining emulsion type based on the different refractive indices of oil and water. The principle involved is that a parallel bundle of light passing through a globule of oil in water will be converged, whilst passage through a globule of water in oil would cause it to diverge.

Little scientific work has been published on margarine as a colloid system, although the present writer has summarised the data available in several papers.[2] The patent literature reveals some interesting features in colloid chemistry. Thus, Blichfeldt [3] was granted a patent for the idea that a stable margarine emulsion of the O/W type resulted when the oil phase is slowly run into the aqueous phase with constant agitation. This is fundamentally sound. Whilst various water-soluble colloids, *e.g.*, gelatin, egg-yolk, casein, have been used to prepare the initial margarine O/W emulsion, margarine itself is usually of the opposite emulsion type, *i.e.*, W/O. This is a result of emulsion inversion due to subsequent "rolling" or kneading of the chilled granules or flakes into a coherent plastic mass. This "mechanical" inversion is also responsible for butter being a W/O system.

Some workers have aimed at preparing margarines which throughout their preparation are W/O emulsions. Ayres [4] has patented the use of coconut oil stearin as an oil-soluble colloid capable of promoting the dispersion of water in oil. In this case the amount of stearin added, and the temperature of emulsification, are so adjusted that the stearin is present as a solid suspension, and it is, of course, much more easily wetted by oil than by water. The idea is scientifically excellent.

Schou [5] proposes to oxidise a refined vegetable oil, *e.g.*, soya-bean oil, at 250° C. until gelatinisation sets in. The product is cooled to 100° C. and mixed with twice its volume of cold soya-bean boil. This "gelatinated" oil, marketed as a thick syrupy liquid, is an excellent agent for preparing water-in-oil emulsions. About 1 per cent. added to the melted fatty contents of a margarine mixture permits the emulsification of about 70 per cent. by volume of water or milk. The aqueous phase is, of course, run into the oil phase, with constant stirring. The probable explanation of Schou's oil so functioning is that partial oxidation has resulted in the molecular structure developing *polar* groups. No investigation concerning

[1] *Chem. Weekblad.*, **26,** 413 (1929).

[2] *Trans. Faraday Soc.*, **16,** Appendix, p. 23 (1921); *Kolloid-Z.*, **28,** 202 (1921); *Z. deut. Öl.Fett-Ind.*, **46,** 321 (1926); Alexander's "Colloid Chemistry" (New York, 1931), Vol. 3, Liesegang's "Kolloidchemische Technologie" (Dresden and Leipzig, 1931), p. 862.

[3] Brit. Pat., 4,505 (1912).

[4] U.S. Pat., 1,467,081 (1923).

[5] Brit. Pats., 178,885; 187,298; 187,299 (1922). See Smith, *Quart. J. Pharm. Pharmacol.*, **3,** 373 (1930).

this change in a vegetable oil seems to have been made, although the subject is of great interest to the colloid chemist.

The work of Pick [1] suggests that the nature of the oil or fat is itself an important factor in determining the permanency of the W/O emulsion. By determining the electrical breakdown of such emulsions Pick observed that hardened fish oils emulsify water best, animal fats less so and vegetable fats least, whether hydrogenated or not. It is interesting to note that the use of hardened oils provides for margarines which hold water very tenaciously.[2]

The actual margarine emulsion in technical practice is exceedingly complex. When the oils and fats are emulsified with "ripened" milk, account must be taken of the influence on the emulsion of the lactic acid, milk colloids and the "curds" present. No systematic investigation has been made along these lines. The effect of salting introduces considerable changes in the degree of dispersion of the water globules in the finished article, a matter which was discussed when considering the salting of butter.

The type of emulsion represented in a margarine should be further investigated in relation to: (1) plasticity and spreading-capacity, (*b*) deterioration in storage (rancidity), (*c*) solution and diffusion of material added in blending, *e.g.*, salt and chemical preservative (where permitted), (*d*) the exudation of moisture, especially in paper-wrapped lots.

It is well-known that although butter is capable of being spread on bread over a fairly wide temperature range, margarine is constantly subject to formulæ alterations depending on the season of the year. The plasticity or spreading-capacity of margarine soon alters with temperature. Oleomargarines containing beef fats and lard more nearly approach butter in physical behaviour. The so-called vegetable margarines soon lose their spreading-capacity below quite definite temperatures depending on their composition.

Undoubtedly, the problem is linked up with the actual differences in chemical constitution, *i.e.*, in the nature of the mixed glycerides of the fatty portion. At the same time physico-chemical investigation of butter and margarines should help to define the physical factors concerned in plasticity. The nature and volume of the external phase and the size-frequency distribution of the dispersed phase will be important factors. The gas content, too, will be important, and it is well-known that over-blending margarines renders them too soft. No data is available regarding margarine, but the gas content of butter is about 10 per cent. by volume,[3] this incidentally being of the same order as the air content of mayonnaise.[4]

[1] *Allgem. Öl-Fettztg.*, **26,** 577 (1929).
[2] Brauer, *Zeits. öffentl. Chem.*, **22,** 209 (1917).
[3] Rogers, U.S. Dept. Agr., Bur. An. Ind., *Bull.*, **162** (1913); Rahn and Storm, *Molkerei-Ztg.* (*Hildesheim*), **37,** 433 (1923).
[4] Epstein and Harris, *Food Inds.*, **2,** 570 (1930).

SALAD DRESSINGS

Salad dressings may be either thin-flowing (salad cream) or viscous (mayonnaise). A salad cream may be made from a variety of ingredients, but mayonnaise is generally understood to be made from eggs, oil, vinegar and flavouring agents. Whilst no ruling has been given concerning the composition of salad creams, the Mayonnaise Products Manufacturers Association of America, Inc., recommended (January 24th, 1928) that [1] : " Mayonnaise, Mayonnaise Dressing, and Mayonnaise Salad Dressing is the clean, sound emulsified product composed of edible vegetable oil, egg yolk or whole egg, a vinegar and/or lemon juice and/or other edible acids, with or without one or more of the following : salt, other condiments, sugar, edible stabilising material. In its preparation are used not less than 50 per cent. of vegetable oil and not less than 6 per cent. of fresh egg solids contained in commercial egg yolk, dried egg yolk or whole egg. In the finished product, the sum of the percentages of vegetable oil and fresh egg yolk free from white is not less than 78 per cent. and the quantity of any stabilising material used does not exceed 2 per cent."

Brooks [2] points out that from a food point of view starchy fillers are undesirable, whilst sugar hastens fermentation and reduces emulsion stability. In his opinion, 40 per cent. of water should be the legal standard for salad creams, and 15 per cent. water for mayonnaise.

Various oils are used in these emulsions, such as olive, sesame, arachis, cotton-seed and maize. Brooks gives the following analyses relating to twelve mayonnaise samples :—

	Percentage.		
	Min.	Max.	Av.
Moisture (H_2O and volatile) .	10·7	15·98	13·19
Total ash	0·59	1·77	1·21
NaCl	0·30	1·41	0·86
Oil and egg-fat . . .	75·13	87·20	81·83
Acetic acid . . .	0·26	0·60	0·41
Nitrogen (ex egg and mustard)	0·19	0·44	0·27
Protein (N × 6·25) . .	1·19	2·75	1·69
Lipoid phosphoric acid. .	0·02	0·06	0·05

Robinson [3] has published a table of analyses furnished by the United States Bureau of Chemistry, from which it is evident that commercial samples vary enormously in composition :—

[1] *Proc. Third. Ann. Convention, Mayonn. Prod. Manfr. Assoc.* (1928), 18.
[2] *The Canner* (1927), p. 39, " Critical Studies in the Legal Chemistry of Foods " (New York, 1927), p. 145 *seq.*
[3] *Am. Food. J.*, **19,** 187 (1924).

Table 35. Analyses of Salad Dressings

Labelled as	Non-Aqueous.	Ash.	Salt.	Reducing Sugar.		Sucrose.	Acid as Acetic.		N_2.	Oil.	Lecithin Phosphoric Acid.	Starch.	Starch.
				Before Inv.	After Inv.		Total.	Volatile.					
Mayonnaise . . .	86·26	1·19	1·09	0·06	0·61	0·61	0·52	0·51	0·207	83·16	0·107	Neg.	
Salad dressing . . .	25·63	3·58	3·32	8·80	10·74	1·84	1·07	0·86	0·278	3·34	0·035	Pos.	3·08
Mayonnaise . . .	56·02	2·74	2·40	1·72	1·78	0·06	1·03	0·75	0·318	46·93	0·085	Neg.	
Mayonnaise . . .	88·37	0·91	0·64	0·30	0·44	0·13	0·41	0·26	0·218	85·22	0·066	Neg.	
Mayonnaise . . .	88·73	0·59	0·30	None	None	None	0·41	0·29	0·261	86·02	0·081	Neg.	
Salad (May.) dressing . .	29·16	2·95	2·26	8·17	11·42	3·09	2·36	2·08	0·736	6·94	0·128	Neg.	
Salad dressing, a mayonnaise	61·15	6·39	5·42	3·45	11·09	7·26	1·45	0·83	0·730	36·48	0·085	Pos.	0·46
Salad May. dressing . .	60·94	3·05	2·40	None	None	None	1·47	1·08	0·593	52·91	0·161	Neg.	
Mayonnaise . . .	59·28	2·35	2·03	None.	None	None	1·26	1·06	0·468	52·83	0·095	Neg.	
Mayonnaise . . .	36·36	3·15	2·73	3·24	12·31	8·62	1·89	1·60	0·374	12·52	0·106	Pos.	4·42
Salad dressing . . .	62·33	3·13	2·65	0·07	0·07	None	1·18	0·89	0·547	54·68	0·145	Neg.	

Salad creams and mayonnaise preparations are always of the O/W type, as would be expected from the fact that egg is used as the principal emulsifying agent. Until recently very little research had been made into the colloidal character of these emulsions, but increasing attention is being given to the use of egg-yolk and egg-albumen and to the type of machinery employed in emulsification. Much remains for investigation. No quantitative data is available regarding the emulsifying efficiencies of egg-yolk, egg-albumin and mustard.

The present writer believes that in place of empirical quantities, investigation should be made concerning the amount of emulsifying agent adsorbed at the oil-water interface. This is related to the degree of dispersion reached in the emulsion, and this, of course, is intimately related to the mechanical conditions employed. Interesting in this connection is the following statement [1] by the Standard and Research Committee of the Mayonnaise Products Manufacturers Association regarding the possibility of using some type of Colloid Mill in the Manufacture of mayonnaise with a view to increasing the degree of dispersion of the oil particles and so improve the consistency and stability of the emulsion. "Three distinct types of Colloid Mills were investigated with the result that none of the three was found suitable for making a mayonnaise emulsion conforming to the proposed standards. Good results were secured on products of the salad dressing or fluid variety containing 50 per cent. or less of oil. With higher percentages of oil, emulsification was not satisfactory. In some cases, the Colloid Mill even tended to break down whatever emulsion was formed. The conclusion of the Committee with regard to Colloid Mills as applied to manufacture of mayonnaise is, therefore, that of the types of mills studied, none are suited to the production of a mayonnaise emulsion conforming to the proposed standard, although they may be serviceable in the case of salad dressing."

No explanation has been given for the failure of the Colloid Mills to furnish a stable emulsion. It is the present writer's opinion that two lines of investigation demand inquiry in this connection: (1) What is the minimum amount of egg-yolk or other emulsifying agent required to stabilise a given interfacial area between oil and water? The original emulsion may be stable because the degree of dispersion is such that *excess* of emulsifying agent is present in the aqueous phase; increasing the fineness of dispersion may produce so enormous an interfacial area that there is insufficient emulsifying agent available to "protect" all the oil globules and the emulsion separates oil. In fact, this method could be used to determine the thickness of adsorbed films and thus check up with the values given by other work based on interfacial tension measurements as

[1] *Proc. Third Ann. Convention, Mayonn. Prod. Manfr. Assoc.* (1928), p. 19.

influenced by adsorption [1]; (2) to what extent does aeration denude the oil globules of emulsifying agent owing to adsorption at the gas/liquid interface, formed by passing the emulsion through a Colloid Mill? The Gaulin homogeniser and other makes of homogenisers (Viscolisers) do not aerate the product and do not lead to breaking of emulsions, although they attain an even greater degree of dispersion of the oil globules.

According to Brooks egg-yolk is preferable to whole egg as an emulsifying agent, since egg-white has only one-fourth the emulsifying capacity of the yolk. Robinson [2] found that frozen egg yolk served to prepare stable emulsions, but they were unstable in transport. Dried egg yolk is also a good emulsifying agent,[3] especially if initially dispersed in water at pH = 8·6, *i.e.*, using alkali. A special egg-yolk preparation for salad creams contains glycerol and acetic acid.[4] Robinson reports that a mixture of powdered egg-albumin, milk albumin and gum arabic permitted very stable emulsions particularly adapted to withstand very low temperatures.

Whether the use of *mixtures* of different emulsifying agents is sound is a question which the present writer believes to require investigation. No work has been published on the relative quantitative adsorption of different emulsifying agents present in an emulsion mixture. The character of the adsorbed envelope around the globules would presumably be influenced by the presence of more than one emulsifying agent being adsorbed. It may quite possibly prove advantageous to confine the emulsifying agent to one ingredient only.

Mark,[5] adopting intermittent emulsification of 100 secs. beating and 10 secs. rest interval, found that "for 10 volumes of egg, 3 or less volumes of oil were always emulsified, while 4 or more volumes never were." Having now obtained this primary emulsion he studied the volumes of oil which could be beaten in successively. They were 4, 7 and 12 volumes respectively. Working in this way it was immaterial whether a salad dressing was prepared with all ingredients at 55° C. or 0° C., and constant beating was as efficient as intermittent agitation when these volumes were adhered to.

An interesting report [6] on a Co-operative Research on Mayonnaise was published in 1924. Irregular results were obtained relating to the amounts of different vegetable oils, which could be emulsified into 15·6 gr. of egg yolk, although the evidence showed that less Italian and Californian olive oils were emulsifiable than Spanish or

[1] *Vide* Clayton, *loc. cit.*, p. 132.
[2] *Loc. cit.*
[3] Cobb and Hunt., *J. Am. Leather Chem. Assoc.*, **21,** 18 (1926).
[4] Epstein, U.S. Pat., 1,595,765 (1926).
[5] Paper reprinted in Brooks' "Critical Studies in the Legal Chemistry of Foods" (New York, 1927), p. 254 *seq.*
[6] Reprinted by Brooks, *loc. cit.*, p. 258.

French olive oils or than cottonseed oils. (The general problem of the degree of emulsifiability of various oils under standard conditions with different emulsifying agents offers a field of research practically unexplored.) It was agreed that as eggs aged, their emulsifying efficiency decreased, and after 6 weeks they gave very poor emulsions. Mark reported that 0·3 per cent. gelatin, dissolved in the vinegar, assisted emulsification, and the resulting emulsions were more stable in transport and storage.

Intermittent agitation is favoured by several of the investigators (Smith, Whitacre and Mark). Regarding the speed of emulsification, preliminary results by Whitacre point to the relationship that " the time, or rather the total number of the revolutions is proportional to the cube of the volume of the oil added." All the data point to the advantage of beating-in a very slow and steady stream of oil rather than successive portions.

The effect of diluting egg with vinegar was also investigated. Again the results are incomplete, but if 1 volume of egg is diluted with 8 volumes of vinegar about 40 volumes of oil can be dispersed.

According to Bitting,[1] who prepared emulsions by shaking by hand and stored them for 90 days for examination of stability, the following are the ratios for stable emulsions :—

Cottonseed Oil.	Vinegar.	Egg-yolk.
74	26	30
76	24	22·5
78	22	15
80	20	10
82	18	5
84	16	2·5

Gray[2] has published several papers dealing with mayonnaise as an emulsion. Discussing the " breaking " of mayonnaise he refers to the two types of trouble familiar with marketed mayonnaise, viz. : (*a*) water separation below the emulsion, (*b*) oil separation above the emulsion. Gray outlines the various causes underlying these separations.

In the first place a correct quantitative relation between the ingredients is demanded, and this within only narrow limits. For instance, in a certain set of experiments with 78 per cent. of oil, 17 per cent. of water and 5 per cent. egg yolk, a permanent emulsion was formed. If the amount of oil was reduced to, say, 76 per cent., the water increased to 19 per cent., the egg remaining the same ; on standing the emulsion separated into two layers—a watery layer below and a creamy layer of emulsion above. If the percentage of oil was increased to 92 per cent., the egg remaining the same, the

[1] *The Canner*, **67**, (15), 32 (1928).

[2] Reprinted as " Mayonnaise and Mayonnaise Products," by the Hazel-Atlas Glass Co., Wheeling, W.Va., U.S.A. (1928).

emulsion also separated on standing, but this time with an oil layer above and the emulsion layer below. If the amount of oil was decreased below 78 per cent. and the water increased, but the egg yolk also increased, the emulsion formed was permanent. In general, the more water in an oil-in-water emulsion the more emulsifying agent is needed to make it permanent.

The other causes of separation enumerated by Gray are : (1) adding the oil too rapidly, whereby dispersion is ineffective, and larger globules are coated with emulsifying agent ; (2) not regulating the conditions of agitation, over-agitation leading to separation (cf. Herschel) ; (3) the effect of heat during storage ; (4) the effect of freezing during storage ; (5) agitation during transport ; (6) bacterial spoilage.

Heating or freezing emulsions which are stabilised by heat-sensitive emulsifying agents very frequently break, owing to the irreversible change in the structure of the agent.

The peculiar effect of agitation during transport is well illustrated by an incident described by Ayres.[1] An emulsion of water in crude petroleum defied separation by chemical or centrifugal methods. Transport in a train effected complete separation. The present writer has seen many examples of emulsions " broken " by transport agitation, and believes the subject would repay investigation of the effect of ordered agitation on oil separation.

Gray and Southwick [2] have studied the consistency and stability of mayonnaise employing the Gardiner mobilometer.[3] Excellent photo-micrographs of the emulsions studied accompany their results, but the present writer does not believe that the use of the mobilometer is sufficiently indicative of consistency. The method is to note the time taken for a weighted plunger to fall a given depth through the emulsion. Repeated readings on the same sample differ, the emulsion coarsening, and ultimately even breaking, as the number of times the plunger falls is increased. The authors draw their conclusions from the number of plunges required before either a steady time reading is given or breaking occurs. A much more satisfactory method would be to measure viscosity in an apparatus of the MacMichael type, maintaining a number of samples from the same batch of emulsion at constant temperature for determining viscosity change with time.

TRANSPARENT EMULSIONS

Flavourings are frequently made up in emulsion form,[4] and a recent investigation by Whitmore and Linehan [5] on the emulsifica-

[1] *J. Soc. Chem. Ind.*, **35,** 678 (1916).
[2] *Food Inds.*, **1,** 300 (1929).
[3] *Ind. Eng. Chem.*, **19,** 724 (1927).
[4] Wesley, *Confectioners J.*, (May 1927), p. 89.
[5] *Ind. Eng. Chem.*, **21,** 878 (1929).

tion of essential oils has some interesting features, inasmuch as they simulated alcoholic solutions by preparation of *transparent* emulsions.

Transparent emulsions are possible when the two liquids concerned have the same values for the refractive index and for the optical dispersive power. Thus, glycerol gives transparent emulsions in a solution of calcium oleate in CCl_4. The production of transparent emulsions has been made the subject of a patent by Rector,[1] who proposes to make medicinal emulsions (*e.g.*, of cod-liver oil) transparent by bringing the refractive indices of the phases into closer similarity through the addition of salt or sugar. Transparent emulsions transmit the complete spectrum ; a complete optical study has been reported by Sogani.[2]

The essential oil emulsions referred to contained 5 per cent. by volume of orange, lemon, lime or peppermint oil, dispersed in an aqueous solution of gum arabic, gelatin, agar or other colloid, and the refractive index of the aqueous phase brought to that of the oil by addition of an 80 per cent. invert sugar solution or a mixture of 2 parts of invert sugar and 1 part of sucrose.

When, however, wintergreen, anise or cinnamon oil was required in emulsion the amount of invert sugar necessary to adjust the refractive index of the aqueous phase to the very high value of the oil proved impracticable. Consequently, the authors tried to reduce the refractive index of the oil and succeeded by adding the ethyl esters of coconut oil. Transparent emulsions were then prepared as before with the aid of invert sugar.

As would be expected, temperature changes resulted in loss of transparency ; the refractive indices were the same for the two phases at 25° C., and in the range 20°—30° C., the transparency was unimpaired. Outside that range opacity resulted, since the change in refractive index with change in temperature is different in the aqueous and oil phases.

PARENTERAL ADMINISTRATION OF EMULSIONS

Modern research is concerned more and more with the utilisation of emulsions given parenterally. Experiments on the injection of emulsions for therapeutic purposes are described by Hedvall,[3] using insulin and by Strauch,[4] who stabilised olive oil emulsions with metacholestearin and myricin. Nomura [5] investigated parenteral nourishment by intravenous injection of emulsion,

[1] U.S. Pat., 1,389,161 (1921) ; *Chem. Age* (*New York*), **29,** 408 (1921). See also Fleming, *J. Agr. Research*, **33,** 17 (1926).
[2] *Phil. Mag.*, **1,** 321 (1926) ; Sethi, *Proc. Ind. Assoc. Cult. Sci.*, **6,** 134 (1920—21).
[3] *Acta. med. Scand.*, **62,** 334 (1925).
[4] *J. Am. Med. Assoc.*, **92,** 1177 (1929).
[5] *Tohoku J. Exptl. Med.*, **12,** 247 (1929). Cf. also Locatelli, *Arch. sci. biol.* (*Italy*), **12,** 407 (1928).

whilst Scheffer [1] studied the extent and duration of fat deposition in the lung. The whole question of the fate of intravenously infused emulsions is dealt with by von Bodo and Scheffer.[2]

The early work of Mills [3] concerned subcutaneous injections of emulsions of olive, cottonseed, sesame, arachis and corn oils, lard and human fat into cats, using 10 per cent. oil emulsions. Control tests were made with the oils and fats unemulsified. A group of cats were maintained on a low-protein diet. The absorption of oils or fats was increased from 4—6 times when presented in emulsion form, lard emulsions being especially useful, and about 8 times better absorbed than were the other emulsions. On a high-protein diet, absorption was the same for the clear oils as for their emulsions.

This work was extended by Koehne and Mendel,[4] who employed a cod-liver oil emulsion containing 75 per cent. oil, 23 per cent. water, and 2 per cent. egg lecithin. Subcutaneous, intramuscular and intraperitoneal injections were made into young rats fed on a rickets-producing diet. " Without doubt the rats received considerable protection against rickets from the oil injections. In general, the clear oil was more potent than the emulsion."

The authors raise the question as to a possible connection between the degree of dispersion of an emulsion and its utilisation, but no tests were made on this. The nature of the emulsifying agent would seem to demand special consideration in such therapy, especially for intravenous injections.

Koehne and Mendel aptly summarise the need for investigations along the above lines : " The question whether fat, introduced into the body by ways other than the digestive tract, can be made available for nutritive purposes, has both a scientific and a practical interest. Physiologically it is important to know to what extent the fat molecules so administered must be altered to become suitable for processes of metabolism and to ascertain what capacity the organism possesses for making the requisite changes. Fat has frequently been introduced parenterally, both in animal experiments and clinically, as a seemingly favourable solvent for drugs, or as a vehicle for substances of diagnostic value in X-ray work. Because of its high potential energy content fat has also been injected at times when it has been found necessary to attempt to nourish patients by other than the customary alimentary means." For more extended treatment the student is referred to a highly interesting paper by Bernhardt and Strauch [5] on " Oil-in-Water and Water-in-Oil Emulsions in Their Relation to Medicine."

[1] *Arch. exptl. Path. Pharmakol.*, **124,** 334 (1927).
[2] *Ibid.*, **124,** 326 (1927).
[3] *Arch. Internal Med.*, **7,** 694 (1911).
[4] *J. Nutrition*, **1,** 399 (1929).
[5] *Z. klin. Med.*, **104,** 723 (1926).

THE CHYLOMICRON EMULSION

In its function of distributing the products of digestion blood carries minute globules of fat, termed by Gage [1] *chylomicrons*. A detailed study of blood as an emulsion was made by Gage and Fish, [2] who fed men and animals with fats and fatty acids mixed with carbohydrates. An hourly examination of the blood was carried out during the digestive cycle and a count made in the dark-field of the chylomicrons, these measuring 1—5μ. The chylomicron content of the blood was found to increase in from $\frac{1}{2}$ to $1\frac{1}{2}$ hours after the meal, whilst in 6—10 hours the chylomicrons had vanished, the time of vanishing varying with the kind and quantity of fat fed.

The subject has quite recently been given attention by Ludlum, Taft and Nugent,[3] who were particularly interested in the nature of the protective film around the globules. Determinations of the isoelectric point of the chylomicrons in human serum were made by a maximum flocculation and a cataphoretic method, and the values invariably lay between pH 4·6 and 5·4, *i.e.*, between the accepted values for the isoelectric points of serum albumin and serum globulin. There is evidence that the isoelectric point lies between pH 4·8 and 5·0. Presumably, therefore, the chylomicrons are surrounded by films of protein, *viz.*, a mixture of serum albumin and serum globulin.

The importance of this observation in physiology is its bearing on the method of assimilation of food fat from the blood and its storage and eventual utilisation. Assuming that the chylomicrons are taken up as such by cells, the physico-chemical character of the surface films assumes primary importance. As the authors point out, acid-fast bacteria with their lipoid-like surfaces are only readily phagocytised when they are treated with the proper serum, which forms a protein film around them. By analogy, the removal of fat from the blood probably depends upon the chylomicrons possessing a surface film of protein which permits wetting by the material of the assimilating cells.

Again, the fat in storage tissues is frequently found as globules much larger than chylomicrons. It is possible that coalescence of the chylomicrons has been brought about by the cell tissues, in which case there is involved the destruction of the protective protein film, possibly by the action of proteolytic enzymes in the cell.

MEDICINAL PARAFFIN EMULSIONS

Medicinal paraffin is frequently prescribed in cases of intestinal stasis, mucous colitis and post-operative treatment. Such paraffin

[1] *The Cornell Veterinarian*, **10,** 154 (1920).
[2] *Am. J. Anatomy*, **34,** 1 (1924).
[3] *Colloid Symposium Annual*, **7,** 233 (1929); *J. Phys. Chem.*, **35,** 269 (1931).

is readily emulsified by the stomach juices, as observed by Schlagintweit [1] in his experiments with white mice. Its action depends partly on the capacity for holding water in emulsion, *e.g.*, stools containing paraffin analysed about 15 per cent. more water than corresponding non-paraffin stools. There is thus an actual change in the consistency of the intestinal contents.[2]

The former idea of lubrication being practically discarded in favour of the action of paraffin in softening and moistening the stool, it is increasingly the practice to administer a paraffin emulsion. Agar is commonly used as the emulsifying agent.[3]

The *emulsio petroli*, Brit. Pharm. Codex, uses gum tragacanth [4] as emulsifying agent, but Knight [5] finds that gum acacia produces smaller globules, although he recommends agar as cheaper.

EMULSIONS IN BREADMAKING

Fat and oil have long been used in breadmaking and biscuit manufacture to effect modification of the gluten, making this less tough and more readily softened during fermentation. Tests with the Chopin apparatus prove that a dough containing fat has a reduced tenacity.[6] Obviously, intimate admixture of the fat or oil is desirable, an end readily achieved if the fat or oil is presented in emulsion form.

Various patents have been granted for emulsion shortening agents. Thus, Baker [7] emulsifies 45—60 per cent. lard using sodium stearate and stearic acid, whilst Pæssler [8] employs 10 per cent. by weight of the edible oil of sodium or ammonium stearate as emulsifying agent. Clark [9] proposes Irish moss. Archibald [10] emulsifies cottonseed oil in water, using saponin and glycerin or Irish moss. To improve the quality and yield of bread, Matti [11] recommends that an emulsion of paraffin (55 per cent.), glycerin (35 per cent.), and water (10 per cent.) be added to the dough in the proportion of 0·3 per cent. of the weight of flour. Dunham [12] has several patents dealing with emulsions, wherein 20—70 per cent. coconut oil is emulsified with wheat flour or other starchy material. The emulsion is then homogenised and dried.

The function of such emulsion in breadmaking has been investi-

[1] *Pharm. J.*, **119,** 461 (1927).
[2] Schlagintweit, *Arch. exptl. Path. Pharmakol.*, **124,** 59 (1927).
[3] Miller, Canadian Pat., 277,180 (1928); U.S. Pat., 1,605,130 (1926).
[4] Schulek and Vastagh, *Pharm. Zentralhalle*, **69,** 275 (1928).
[5] *Pharm. J.*, **121,** 297 (1928).
[6] Casaubon, *Bull. assoc. chim. sucr. dist.*, **47,** 44 (1930).
[7] U.S. Pat., 1,553,294 (1925).
[8] U.S. Pat., 1,203,905 (1916).
[9] U.S. Pat., 1,491,830 (1924).
[10] Brit. Pat., 330,779 (1929).
[11] U.S. Pat., 1,750,720 (1930).
[12] Brit. Pat., 169,493 (1920); U.S. Pat., 1,431,938 (1923) and 1,432,057 (1923).

gated by Bennion,[1] who later extended his study to confectionery. One per cent. and 5 per cent. of arachis oil in water were employed, without emulsifying agent, and 11 c.c. of the mixture were incorporated in 20 gr. of various flours, whereupon an analysis was made for gluten. " If flour is treated with ether or any other fat solvent in order to remove the fat, it then becomes almost impossible to make a dough suitable for breadmaking purposes. It seems that the fat contained in the flour naturally, has some effect on the formation of gluten in a dough." The admixture of arachis oil to flours increased the gluten as determined by analysis, and the emulsions did the same to a slightly less extent. It was noticed that all the glutens obtained from doughs in which emulsion had been incorporated were much softer and more elastic and on drying at 105° C. were creamy in colour, thus being superior to the colour of dried ordinary gluten (brownish or greyish). There is " a mellowing effect of the gluten, making it capable of possessing greater elasticity without impairing its strength."

The bread made from doughs containing emulsion gave whiter crumb. It was observed that during the proving of the loaves, the emulsion samples proved the most slowly, but the lesser volume when proving was completed was counterbalanced by the increased oven-spring, probably because of the now greater elasticity of the gluten. With gluten breads, emulsions gave marked effects, the colour being more creamy, the vesiculation much more even and the sheen greatly improved. Although oil in bread keeps the crumb moist, the use of an oil emulsion will maintain the bread moist for a longer time.

For breadmaking Bennion uses a 33 per cent. oil emulsion, 2 quarts to the sack for ordinary bread, 1 gallon to the sack for milk and Vienna breads.

In confectionery articles, where only a small amount of fat is employed, the advantage of using an emulsion becomes obvious, the ordinary rubbing of fat into flour being inefficient. As milk is usually an ingredient, it serves admirably as the emulsifying agent. Usually, emulsification is conducted at about 75° C. and the emulsion run over a cooler. A suitable emulsion can be made by dispersing 5·25 lb. of oil or fat in 1·5 gallons of water containing 1·5 lb. of milk powder. Bennion advises that the proportion of fat to flour is 3 oz. to the 1 lb., and the corresponding volume of emulsion will greatly improve the bloom and general appearance of sultana scones, rock cakes, rice buns, lemon buns and coffee buns.

Bennion's work has been recently examined by Van Dyk [2] using Dutch flour. He found no appreciable increase in the amount of gluten. With an English flour (Millenium) and Wesson cooking oil,

[1] *J. Soc. Chem. Ind.*, **45,** 435 T (1926) ; *Food Manfr.*, **4,** 247 (1929).
[2] *J. Soc. Chem. Ind.*, **49,** 421 T (1930).

a small increase in the amount of gluten was observed. Apparently, the rate of drying of the gluten influences the results. However, Van Dyk confirms that a softer and more elastic gluten was obtained from those doughs which had been made up with emulsion.

SHORTENING AGENTS

In such diverse products as bread, soda crackers, sugar cookies, drop cakes, pound cakes and biscuits, it is common to use butter, lard, lard compound, vegetable fats and hydrogenated oils as shortenings.[1] Their function is to make the product more tender and break easily, but the quantitative influence of the various oils and fats differs considerably.

Platt and Fleming [2] have defined a shortening as "any fat or fixed oil used as an ingredient in baked products" and "that material has the greatest shortening power which, when baked in a dough under standard conditions, gives to the product a minimum breaking strength and a minimum crushing strength."

Davis devised a machine for the quantitative determination of the breaking strength of sugar cookies containing different shortenings, working to a standard recipe and under controlled conditions. Lard was found to be the best shortening, whilst hydrogenation of oils increased their value as shortenings.

Platt and Fleming have studied the physico-chemical aspects of the action of shortenings, observing that even vaseline can function. Microscope examination of stained dough and cooked products showed that the shortening was present as thin layers between the starch granules and sometimes enveloping them. The shortening breaks up the dough or cookie and "forms innumerable, minute fat-filled cracks or breaches of continuity throughout the structure of the biscuit, so that the biscuit consists of many minute particles, which are separated from the adjoining particles by a thin layer of shortening."

The authors introduce the idea that the shortening capacity of fats varies with the percentage of unsaturated glycerides, although the plasticity of the shortening at the temperature of mixing plays some *rôle*.[3] The greater the percentage of unsaturated glycerides, the more effective is the agent, and it is suggested that the results may fall into line with the Hardy-Langmuir-Harkins theory of wetting and spreading. The problem deserves quantitative investigation along such lines of thought, particularly as unanimity regarding shortening efficiency is not yet established.[4]

[1] Cf. Bailey, *Am. Food J.*, **19,** 369 (1924); Bosart, *ibid.*, **19,** 481 (1924).
[2] *Ind. Eng. Chem.*, **15,** 390 (1923); cf. Davis, *ibid.*, **13,** 797 (1921).
[3] Platt, *Baking Tech.*, **2,** 216 (1923).
[4] *Vide* Davis, *Ind. Eng. Chem.*, **15,** 1089 (1923); Graff, *ibid.*, **20,** 1312 (1928).

CHAPTER VII

THE COLLOID CHEMISTRY OF MILK AND MILK PRODUCTS

MILK

Cow's milk, a secretion of the mammary gland, offers an interesting field for study by the colloid physicist and chemist, extending also to its products, such as cream and butter. Other milks, such as human milk, present similar problems, but the bulk of the physico-chemical research has centred round cow's milk. Unless otherwise stated, the term " milk " is understood to refer to cow's milk.

Milk has a density of 1·029—1·033, and freezes at −0·5345° C. (ranging from −0·558° to −0·514° C.).[1] Its electrical conductivity [2] varies from $K_{15} = 0{\cdot}0035$ to $K_{15} = 0{\cdot}0047$, being usually between 0·0040—0·0042. There is a marked correlation between conductivity and chloride content of milk.[3] The recent work of Strohecker [4] gives the conductivity as $45—50 \times 10^{-4}$, observed values being $42{\cdot}7—74{\cdot}4 \times 10^{-4}$; values above 50×10^{-4} are abnormal.

Fricke [5] has discussed the general question of the electrical conductivity of emulsions. He points out that conductivity measurements may give values for (*a*) the specific conductivity, (*b*) the concentration, or (*c*) eccentricity of form of the particles of the dispersed phase. Assuming suspended non-conducting homogenous spheroids, the following formula is derived :—

$$\frac{K - k}{2K + k} = \frac{C}{2}$$

where k = specific conductivity of the emulsion.
K = ,, ,, ,, continuous (external) phase.
C = volume concentration of the dispersed (internal) phase.

[1] Monier-Williams, " The Freezing Point of Milk," Food Report 22 (London, H.M. Stationery Office, 1914). *Vide* also Bailey, *J. Assoc. Official Agr. Chem.*, **6,** 429 (1923); Hortvet, *Ind. Eng. Chem.*, **13,** 198 (1921); Pritzer, *Z. Untersuch. Lebensm.*, **34,** 69 (1917).

[2] Cf. Gerber, *Z. Untersuch. Lebensm.*, **51,** 336 (1926); **54,** 257 (1927); Koeppe, *Jahrb., Kinderheilk.*, **47,** 389 (1898); Roadhouse and Koestler, *J. Dairy Sci.*, **12,** 426 (1929); Dannhofer and Mojer, *Molkerei-Ztg.*, **14,** 149 (1930).

[3] Coste and Shelbourn, *Analyst*, **44**· 158 (1919).

[4] *Z. Untersuch. Lebensm.*, **49,** 342 (1925). Cf. Palmer and Dahle, *J. Dairy Sci.*, **5,** 7 (1922); Krenn, *Z. Untersuch. Lebensm.*, **59,** 32 (1930).

[5] *Phys. Rev.*, **24,** 575 (1924).

Fricke and Morse [1] experimentally verified the formula, working with cream, values of C being up to 62 per cent.

Non-conducting substances in milk, by obstructing the passage of ions, reduce the electrical conductivity. Thus, Taylor [2] observed an increase in the conductance of milk of 11·4 per cent, when the fat (5 per cent.) was removed. According to Jackson [3] the concentrating of milk, *e.g.*, preparing condensed milk, leads to a maximum conductance when the total solids = 28 per cent., proteins being 8 per cent. With sweetened condensed milk, the maximum conductance is quickly reached, and rapidly decreases after the protein concentration reaches 4 per cent. Wardlaw [4] found that removal of fat increased the electrical conductivity of milk, whilst Quagliarello [5] finds that cooling milk increases the viscosity but does not affect conductivity. Fürth [6] has measured the dielectric constants of milks and gives the values : human 75, cow 66.

Until recently, singularly little work had been reported relating to the surface tension of milk. Carapelle and Chimera [7] assert that the surface tension varies inversely as the fat content of the milk, whereas Behrendt [8] states that fat has no influence on the value, but that proteins and fatty acids are the determining factors. According to Dahlberg and Hening [9] the surface tension of milk decreases with increased fat content and with ageing, whilst pasteuring raises the value. Freezing, as expected, decreases the surface tension.[10] Kopaczewski [11] gives the surface tension of milk at 15° C. as 52·8 dynes/cm. The table on p. 205, due to Dahlberg and Hening,[12] shows surface tension results (Du Noüy method) as influenced by fat content, pasteurisation and age.

Surface tension phenomena are of fundamental importance in dairy chemistry, and will be discussed in some detail later (see p. 227).

Milk is slightly acid when freshly-drawn, and this acidity is independent of bacterial activity, which later may increase the acidity to a considerable extent. Electrometric determinations of the pH value of milk have been made by Faber and Hadden,[13] who give the value 6·89. Other authors report pH values as 6·6 (Clark),[14]

[1] *Ibid.*, **25,** 361 (1925).
[2] *J. Proc. Roy. Soc. N.S. Wales*, **47,** 179 (1913).
[3] *J. Soc. Chem. Ind.*, **33,** 59 (1914).
[4] *Proc. Linnean Soc., N.S. Wales*, **43,** 613 (1918).
[5] *La Pediatra*, **24,** 1 (1919).
[6] *Ann. Phys.*, **70,** 63 (1923).
[7] *Rev. d'Hygiene Med. Infantiles*, **9,** 167 (1910).
[8] *Z. Kinderheilk*, **33,** 209 (1922).
[9] New York Agr. Expt. Sta., *Tech. Bull.*, **113,** 1—42 (1924).
[10] Quagliarello, *La Pediatra*, **24,** 1 (1919) ; Mohr and Brockmann, *Milchwirtschaft Forsch.*, **10,** 93 (1930).
[11] " L'Etat Colloidal et L'Industrie," Vol. I. (Paris, 1925), p. 134. See also, Burri and Nussbaumer, *Biochem. Z.*, **22,** 90 (1909) ; Bauer, *Biochem. Z.*, **32,** 362 (1911).
[12] N.Y. Agr. Expt. Sta., *Tech. Bull.* **113** (1925). Cf. also Mohr and Brockman, *loc. cit.* **10,** 72 (1930), who give extensive data.
[13] *Med. Clin. N. America*, **6** (2), 245 (1922).
[14] " Determination of Hydrogen Ions " (London, 1928), p. 104.

TABLE 36. SURFACE TENSION OF MILK

Fat.	Surface Tension at 3° C.			
	Raw.		Pasteurised.	
	1st Day.	3rd Day.	1st Day.	3rd Day.
Per cent.	Dynes/cm.	Dynes/cm.	Dynes/cm.	Dynes/cm.
0	57·41	57·73	59·33	59·32
5	55·32	52·69	55·83	56·41
10	52·83	51·50	54·75	54·79
20	51·78	50·54	53·44	52·59
25	50·43	49·36	50·62	51·65
30	50·01	48·77	50·55	50·91
35	49·12	47·33	50·23	50·47

6·54 (Duncombe),[1] 6·8 (Taylor),[2] 6·6 (Davidsohn),[3] 6·5—6·6 (Baker and Breed),[4] 6·6—6·8 (Milroy),[5] 6·7—6·8 (Schultz),[6] 6·5—7·2 (Baker and van Slyke).[7]

Tillmans and Obermeier [8] believe that the acidity of fresh milk is due to the presence of acid phosphates, and Comel [9] points out that the buffer effect is similar to that of a M/30 phosphate mixture. It is known that the solubility of calcium phosphate in milk is affected by the concentration of citrates present.[10] Carbonic acid and carbonates also play a part.[11] The buffering action of milk is a maximum at pH 5·50. Now casein has a buffer action over the pH range 4·5—5·7, with a maximum at pH 5·2; it is believed, therefore, that casein is a prime factor in the buffer action of milk over this zone of acidity.[12] Buchanan and Peterson,[13] however, could find no marked change in the buffer value of casein over the iso-electric range, pH 4·5—5·0, and they believe that phosphates in milk constitute the most important buffers, a view supported by the marked similarity between the titration curves of milk and Na_2HPO_4.

[1] *J. Dairy Sci.*, **1,** 86 (1924).
[2] *J. Proc. Roy. Soc. N.S. Wales*, **47,** 174 (1913).
[3] *Z. Kinderheilk*, **9,** 11 (1913).
[4] *J. Biol. Chem.*, **43,** 221 (1920).
[5] *Biochem. J.*, **9,** 215 (1915). See also Roadhouse and Koestler, *J. Dairy Sci.*, **12,** 426 (1929).
[6] *J. Dairy Sci.*, **5,** 383 (1922).
[7] *J. Biol. Chem.*, **40,** 345 (1919). See also Evans, *J. Physiol.*, **54,** 353 (1921); McInerney, *J. Dairy Sci.*, **3,** 227 (1920).
[8] *Z. Untersuch. Lebensm.*, **40,** 345 (1919).
[9] *Arch. ital. biol.*, **81,** 61 (1929).
[10] Whittier, *J, Dairy Sci.*, **12,** 405 (1929).
[11] Van Slyke and Baker, *J. Biol. Chem.*, **40,** 335 (1919).
[12] Whittier, *J. Biol. Chem.*, **83,** 79 (1929).
[13] *J. Dairy Sci.*, **10,** 224 (1927).

The whole problem is exceedingly complicated, and one must agree with Comel [1] that the buffer action of milk is due to the presence or formation of complex acid salts, proteinates and free proteins. Clark [2] has discussed the question in a most valuable article : " The Acid-Base Equilibria of Milk." Critically reviewing the data available, Clark points out that citrate, phosphate and bicarbonate, together with proteins, act as buffers in milk. The presence of calcium, forming salts of low solubility, complicates the system. " No thoughtful person will take very seriously some of the conclusions regarding the nature of the equilibria in milk until the conduct of calcium-phosphate-citrate systems, in the absence and in the presence of such proteins as are found in milk, shall have been established on a quantitative basis."

The colloid chemistry of milk deals with those substances, such as casein and lact-albumin, which are inherently colloidal in nature, and with the materials which behave as colloidal systems by virtue of their fine state of subdivision, *e.g.*, the fat present as emulsion, the cell-content of milk, and milk enzymes.

The average composition of various milks as given by Leach's compilation from Koenig's " Chemie der mensch-Nahrung und Genussmittel " is :—

Constant.	Cow.	Human.	Goat.	Ewe.	Mare.	Ass.
S.G. . . .	1·0316	1·03	1·0305	1·0298	1·0347	1·036
Water per cent. .	87·27	87·41	85·71	80·82	90·78	89·64
Casein . .	3·02	1·03	3·20	4·97	1·24	0·67
Albumin . .	0·53	1·26	1·09	1·55	0·75	1·55
Fat . . .	3·64	3·78	4·78	6·86	1·21	1·64
Milk Sugar . .	4·88	6·21	4·46	4·91	5·67	5·99
Ash . . .	0·71	0·31	0·76	0·89	0·35	0·51

THE ASH OF MILK

As the ash constituents of milk, particularly the calcium content, assumes considerable importance in relation to the coagulation of milk by rennin and to thermal changes, the composition of the ash deserves attention. Table 37 collects the ash analysis as given by Clark [3] in his assumed composition of milk and by Roadhouse and Koestler [4] in their account of the milk of three different cows, which gave especially good tasting milk. The results are given as grams per litre.

Rossi [5] has subjected milk to ultrafiltration through 6 per cent. collodion membranes, finding that about 60 per cent. of the Ca. and

[1] *Bull. soc. biol. ital. sper.*, **3,** 908 (1928).
[2] In " Fundamentals of Dairy Science " (New York, 1928), Chap, VI.
[3] *J. Dairy Sci.*, **10,** 197 (1927).
[4] *Ibid.*, **12,** 426 (1929).
[5] *Boll. soc. ital. biol. sper.*, **5,** 475 (1930).

TABLE 37. THE ASH CONSTITUENTS OF MILK

	Clark.	Roadhouse and Koestler.		
CaO . .	1·78	1·582	1·579	1·911
MgO . .	0·30	0·035	0·121	0·173
K_2O . .	1·80	1·982	1·957	1·881
Na_2O . .	0·72	0·380	0·579	0·684
Cl . . .	1·00	0·933	0·832	0·836
SO_3 . .	0·11	0·192	0·235	0·303
P_2O_5 . .	1·50	1·960	2·018	2·324
Citric Acid .	2·00	2·62	2·69	2·79

P. and 18 per cent. of the K. were non-diffusible, whilst all the Cl. was diffusible. Pasteurisation of the milk had no influence on the distribution of the electrolytes in the milk and in the ultrafiltrate.

A fifteen-hour dialysis of milk with a collodion filter against distilled water at 15° C. was made by Roadhouse and Koestler, who give the following table of their results :—

TABLE 38. ANALYSIS OF DIALYSED MILK.

Determination.	Original Milk.	Residue after Dialysis.	Dialysate.
Specific weight . . .	1·0306	1·0168	1·009018
Fat (per cent.) . . .	3·69	3·30	—
Dry matter (per cent.) . .	12·32	8·60	2·10
Lactose (per cent.) . .	4·76	2·31	1·73
Chloride (per cent.) . .	—	0·041	0·0195
Ash (per cent.) . . .	—	0·0433	0·0372
P_2O_5 (per cent.) . . .	—	0·110	0·022
CaO (per cent.) . . .	—	0·112	0·024

Citric acid is an important factor in the salt equilibria of milk, and exists mainly as trisodium citrate, and to a small extent as tripotassium citrate. Some authorities believe that calcium and magnesium citrates are also present in milk. Sommer and Hart [1] found the citric acid content of milk to be from 0·232—0·242 per cent., a quantity unchanged by heating even to 118°C. for 60 minutes. No citrate was precipitated under the conditions of their experiments. Sherwood and Hammer,[2] reporting on 335 determinations of citric acid in milk from individual cows of four noted breeds, state that the quantity varies from 0·07—0·33 per cent., with an average of 0·18 per cent.

[1] *J. Biol. Chem.*, **35,** 313 (1918) ; cf. Supplee and Bellis, *ibid.*, **48,** 453 (1921).
[2] Iowa State Coll. Agr. Expt. Sta., *Res. Bull.*, **90,** 19 (1926).

MILK AS AN EMULSION

Milk contains about 3·6 per cent. of butter-fat, present as an emulsion of fair stability. On standing, the greater portion of the fat rises to the surface as CREAM, and the milk then contains about 0·2 per cent. of fat (water = 90·4 per cent.). Much argument has taken place as to whether the fat globules in milk are surrounded by a membrane, or by a gelatinous mucoid, semi-liquid substance,[1] usually referred to under the Danish name "slim-membran." Struve [2] considered the membrane to consist of insoluble casein, whilst Babcock [3] believed it to be milk-fibrin. Schmid [4] in a paper on "The Microscopical Investigation of Cow Milk" found evidence for the presence of a membrane around the globules. Anderson [5] believes that the stability of the fat globules in milk is due entirely to a protective layer of casein.

Hattori [6] claims to have isolated a white powder ("haptein") of a new protein, corresponding with the membrane, the yield being 0·02 per cent.[7]

Whatever the ultimate chemical nature of the membrane around the globules, the idea of such a membrane is quite in accord with general work on emulsions, certain emulsifying agents being so strongly adsorbed at the fat/water interface as to be "precipitated," frequently irreversibly so.[8]

The fat globules in normal milk range from 0·1μ to 10μ. in diameter, the average approximating 3μ. The number is of the order of $2 - 4 \times 10^{12}$ globules per c.c. of milk.[9] The size of the globules varies with several factors[10]:—(1) Breed: Jersey and Guernsey cows give milk wherein the globules are larger than in milk from other breeds. (2) Lactation period: the globule-size decreases with advancing lactation, although the number increases.

[1] Storch, *Beretning frä den Kgl. Vet. og Landbohojskole Lab.*, **36,** 1 (1897). *Vide* also the later work of Voltz., *Arch. ges. Physiol.* (*Pflüger's*), **102,** 373 (1904); Abderhalden and Voltz, *Z. physiol. Chem.*, **59,** 13 (1909); Rosengren, *Milch-Ztg.*, **33,** 337 (1904); Rahn, *Forsch. Geb. Milchw. Molkereiwesens*, **1,** 133, 165, 213 (1921); Bleyer and Kallmann, *Biochem. Z.*, **153,** 469 (1924).

[2] *J. prakt. Chem.*, **27,** 249 (1884); cf. Titus, Sommer and Hart, *J. Biol. Chem.*, **76,** 237 (1928).

[3] *Milch-Ztg.*, **17,** 809 (1888); Richmond, *Analyst*, **22,** 198 (1897).

[4] *Schweiz. Milch-Ztg.*, **50,** 18 (1924).

[5] *Trans. Faraday Soc.*, **19,** 106 (1923); cf. Titus *et al.*, *loc. cit.*

[6] *J. Pharm. Soc. Japan*, No. **516,** 123 (1925); Vol. **49,** 332 (1929).

[7] Hattori and Ogimura, *ibid.*, **49,** 147 (1929).

[8] Clayton, "The Theory of Emulsions and Their Technical Treatment" (London, 1928), p. 120.

[9] Babcock, 4th Ann. Report N.Y. (Geneva) Agr. Expt. Sta. (1885), p. 297; Schellenberger, *Milch. Ztg.*, **22,** 817 (1893); Woll, *J. Agr. Sci.*, **6,** 446 (1892); Stocking, "Manual of Milk Products" (New York, 1917), p. 68 *seq.*; van Dam and Sirks, *Verslag. Land. Onderzoek. Rijkslandbouwproefsta.*, **26,** 106 (1922).

[10] Woll, *J. Agric. Sci.*, **6,** 446 (1892); *Landw. Jahrb.*, **24,** 539 (1895); Hunziker, Purdue Agr. Expt. Sta., *Bull.*, **159,** (1912); Weigmann, *Milchwirtschaft. Forsch.*, **4,** 259 (1927); Holm, "Fundamentals of Dairy Science" (New York, 1928), p. 154 *seq.*

(3) Foodstuff consumed ; small globules result from dry feeds, larger globules from succulent feeds.

The size-frequency analysis of milk emulsion, *i.e.*, the determination of the statistical distribution of the size of the fat globules, has been attempted by several workers.[1] Fig. 23 shows the distribution of fat in whole milk, separated cream and pasteurised cream, the data being obtained by Miss Beckett.[2] The original milk was at 17° C. and was warmed to 62° C. for separating in the creamery ; pasteurisation was conducted at 92° C. Miss Beckett concludes that many large fat globules have been formed during separating and

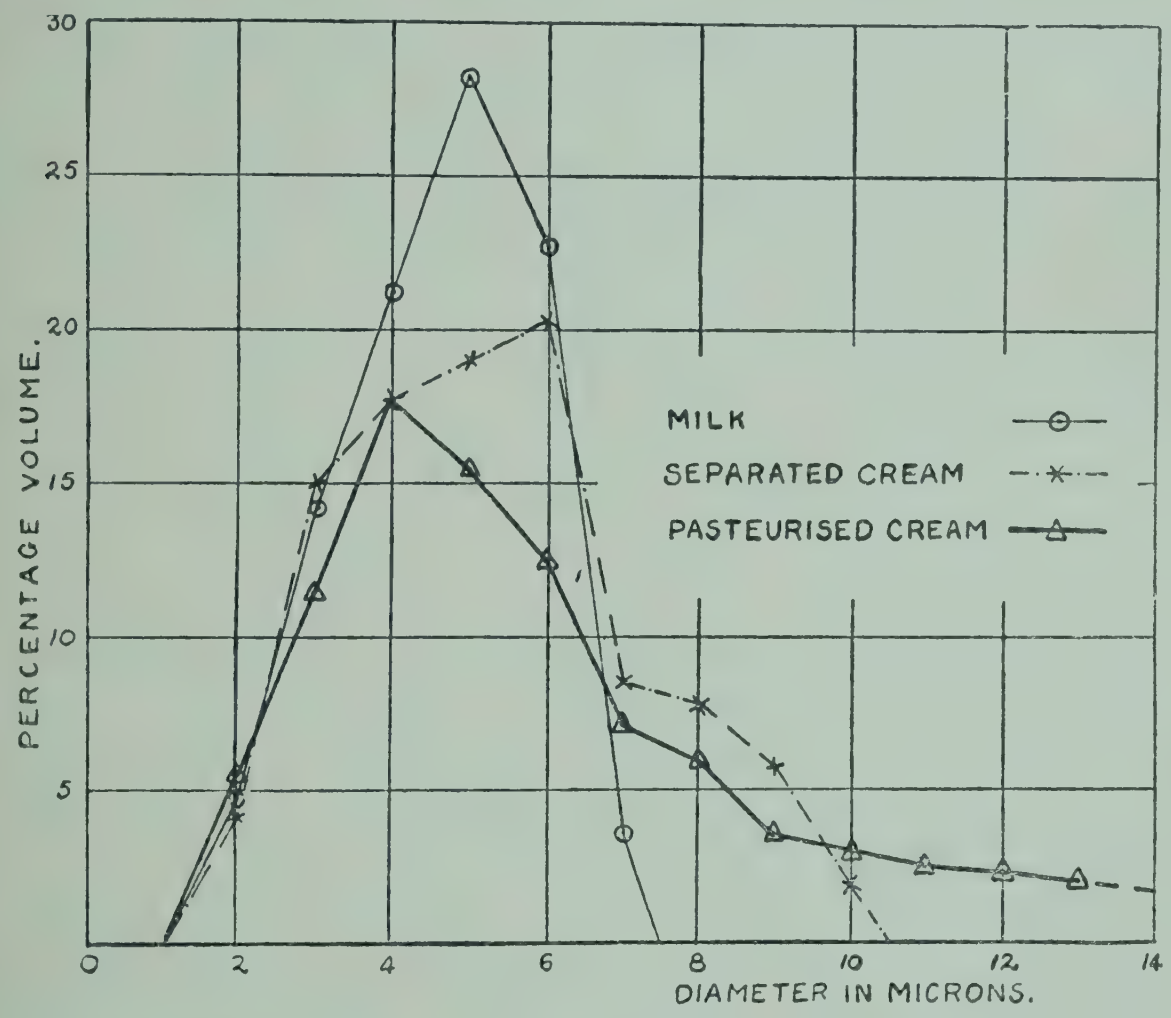


FIG. 23.—Alteration of volume-distribution of fat globules by separation and pasteurisation.

pasteurising whilst the original distribution of small globules remains comparatively unchanged.

Proteins in Milk. Milk contains several proteins, notably casein, lactalbumin and lactoglobulin. Fibrin is not present in normal milk. Casein is by far the most important protein present, in amount as well as its functions. Thus, Høyberg found that the average ratio of casein to other protein = 76·24 for Danish milk, in which the casein content varied from 1·89 to 3·17 per cent.

The electrical behaviour of the colloids in milk has been investigated by Mattson.[3] He found fresh milk to have a pH = 6·6.

[1] Svedberg and Estrup, *Kolloid-Z.*, **9**, 259 (1911) ; *vide* Clayton, " The Theory of Emulsions and their Technical Treatment " (London, 1928), p. 156. Valuable summaries of the work done are given by Rahn and Sharp, " Physik der Milchwirtschaft " (Berlin, 1928), p. 35 *seq.* ; Holm, *loc. cit.*, p. 154 *seq.*

[2] *Econ. Proc. Roy. Dublin Soc.*, **2**, 314 (1927).

[3] *J. Phys. Chem.*, **32**, 1544 (1928).

The proteins on cataphoresis migrated to the anode. Addition of acid, or the incidence of souring, brought about slower anodic migration, with its cessation at pH 4·7. After this pH value the proteins migrated to the cathode. The following table by Mattson illustrates the effect of adding HCl to milk upon the electrical migration of the proteins, using 0·2 c.c. fresh (pasteurised) milk diluted to 20 c.c.

TABLE 39. EFFECT OF HCl ON THE CATAPHORESIS OF MILK PROTEINS.

0·01 N. HCl (c.c. in 20 c.c.).	Flocculation.	μ/sec.	P.D. (millivolts).	pH.
0	—	− 2·92	− 41·2	6·7
0·5	—	− 2·24	− 31·6	5·7
1·0	—	− 1·41	− 19·8	4·9
1·2	++++	± 0	± 0	4·7
1·4	—	+ 1·26	+ 17·7	4·6
1·6	—	+ 1·72	+ 24·3	4·5
1·8	—	+ 2·30	+ 32·4	—
2·0	—	+ 2·47	+ 34·8	—

Casein. Casein is a phosphoprotein but there is some doubt as to it being a single protein or a mixture of proteins.[1] Its molecular weight [2] is probably not less than 12,800 and may even be as high as 192,000. The elemental analysis of casein derived from cows' and human milk respectively is as follows : —[3]

	Cow's.	Human.
C	52·69 per cent.	52·24 per cent.
H	6·81 ,,	7·32 ,,
N	15·65 ,,	14·97 ,,
S	0·832 ,,	1·12 ,,
P	0·877 ,,	0·68 ,,

In milk casein is in combination with calcium being present as a very fine colloidal suspension, the particles varying in diameter from 10—100 μ. Great centrifugal force will not remove casein from milk,[4] but a porous clay filter can hold it back from the serum. The number [5] of casein particles suspended in milk, ranges from $3 - 6 \times 10^{12}$ per c.c. Brownian motion is thus exhibited in milk,[6]

[1] Kondo, *Compt. rend. trav. lab. Carlsberg*, **15,** 1 (1925); Linderstrøm-Lang, *ibid.*, **16,** 48 (1926).
[2] Cohn *et al.*, *J. Biol. Chem.*, **63,** 721 (1925).
[3] Lane-Claypon, " Milk and its Hygienic Relations " (London, 1916), p. 37.
[4] Friedenthal, *Ber.*, **44,** 904 (1911).
[5] Wiegner, *Z. Untersuch. Lebensm.*, **27,** 425 (1914).
[6] Alexander and Bullowa, *Arch. Pediatrics*, **27,** 18 (1910); Bleyer and Seidl, *Kolloid-Z.*, **30,** 117 (1922).

digestion with ether not affecting this, although dilute acid causes coagulation of the particles with consequent cessation of Brownian movement.[1]

The term "caseinogen" is frequent in the literature, and is synonymous with "casein" as described in this text. The older nomenclature reserved the term "casein" for the curd produced by the action of rennin on milk; the modern name for the curd casein is "paracasein."

The physical chemistry of curd casein will be discussed later (p. 278). A debatable point even now is the nature of the existence of casein in milk. Some workers believe it exists as casein lactate.[2] Van Dam demonstrated that union between casein and lactic acid is possible. Using Bredig's ethyl diazo-acetate method, he determined the reduction in hydrogen ion concentration in solutions of lactic acid produced by adding various amounts of casein. The casein combined with a constant amount of lactic acid.

Hammarsten [3] considered that casein is combined with calcium, being in colloidal suspension as calcium caseinogenate. Revis and Payne inferred a combination of casein with calcium phosphate, whilst Richmond [4] suggested that casein existed as a calcium sodium compound in union with one molecular proportion of tricalcium phosphate. According to de Waele,[5] casein in milk is a complex union of calcium casein and phosphate, the composition depending on the pH value prevailing. From pH 7·8—6·5, casein is soluble, but from pH 6·5—4 the amount of calcium in combination and the solubility of the complex progressively diminish.

Van Slyke and Bosworth [6] hold that casein is present in milk as neutral tetracalcium caseinate, while Palmer [7] argues in favour of the calcium caseinate of milk (pH = 6·5) being a mixture of the basic tetracalcium caseinate and a more acid calcium caseinate. Holm [8] points out that the serum from milk contains about 35 per cent. of the milk calcium in soluble form and finds it probable "that the calcium caseinate suspension existing in milk is one containing less calcium than one formed with just sufficient $Ca(OH)_2$ to adjust casein to a pH = 6·6, or the approximate pH of normal milk."

Casein is precipitated from milk by saturating with NaCl, $MgSO_4$ or $(NH_4)_2SO_4$; it is also precipitated by tannin, phosphotungstic acid, copper and zinc sulphate, and rennet. It is not

[1] Kreidl and Neumann, *Arch. ges. Physiol.* (*Pflüger's*), **123,** 528 (1908).
[2] *Chem. Weekblad.*, **7,** 1013 (1910).
[3] *Z. physiol. Chem.*, **7,** 277 (1883); **9,** 273 (1885). See the recent important paper by Moir, *Analyst*, **56,** 2 (1931).
[4] "Dairy Chemistry" (London, 1914), p. 30.
[5] *Ann. physiol. physicochim. biol.*, **3,** 113 (1927); cf. Maynard, *J. Phys. Chem.*, **23,** 145 (1919).
[6] *J. Biol. Chem.*, **14,** 211 (1913); **20,** 135 (1915).
[7] *Ind. Eng. Chem.*, **16,** 631 (1924).
[8] "Fundamentals of Dairy Science" (New York, 1928), p. 140.

coagulated when milk is boiled, but at high temperatures, *e.g.*, 130°—140° C. partial coagulation takes place.[1] Indeed, Jensen and Plattner [2] believe that the browning of milk so heated is caused by the incipient breakdown of the casein.

Preparation of Casein from Milk. In technical practice [3] casein is usually prepared from skim milk, the yield being from 2·75—3·5 per cent. The methods employed are : (*a*) Self-souring or lactic acid precipitation, (*b*) mineral acid precipitation, (*c*) rennet precipitation.

In the self-souring methods, lactic acid develops in the milk owing to the bacterial fermentation of lactose, and the acid solution curdles when warmed to 43°—52° C. with vigorous stirring. The whey is drained from the mass of curd, the character of which depends on the degree of acidity developed. An alternative method is to mix skim milk and an acid whey, both at about 55° C.

The mineral acids used in precipitating casein are either sulphuric or hydrochloric acid. Thus H_2SO_4 (conc. acid diluted with water to 1 in 3) is added to skim milk at 50° C. in the ratio of 1 : 800 with constant stirring. In a few minutes the curd separates and leaves a clear whey. The latter is drained off and the curd washed well with cold water. In contradistinction to this "pressed curd" method, is the "cooked curd" method, wherein after acid precipitation the whey is drained off and the curd is heated in water by means of direct steam to about 79° C., the curd thereby collecting to a plastic, tough mass, which is kneaded to expel water. When using HCl the skim milk is kept within the temperature range 46°—49° C., and commercial HCl (20—23 Bé) gradually added in the ratio : 1 acid to 32 milk, the acid being previously diluted with an equal volume of water. Stirring is essential.

The American large-scale preparation of casein during the world war was based on research [4] into the problem of the acid precipitation of casein from skim milk. HCl was used, as H_2SO_4 leaves a precipitate of $CaSO_4$, which interferes with the subsequent recovery of lactose. The basic idea was that precipitation of casein at its isoelectric point (pH = 4·7) would yield maximum precipitation, and a purer casein free from its compounds except in amount due to occlusion and adsorption. Correct control of acidity, rate of adding acid, and adjustment of temperature, gave the casein in the form of more or less uniformly-sized grains, whence the term "grain-curd casein."

[1] Bardach, *Monatsch.*, **18,** 199 (1897).

[2] *Rev. gén. Lait.*, **4,** 361 (1904/05) ; cf. Conradi, *Münch. med. Woch.*, **48,** 175 (1901).

[3] See Sutermeister "Casein and its Industrial Applications" (New York, 1927), Chap. 3 by Dahlberg ; Tague, "Casein" (London, 1926), Chap. 7 ; Kretschmer, "Die Kaseinerzeugung," reviewed in *Lait*, **11,** 487 (1931).

[4] Clark, *Ind. Eng. Chem.*, **12,** 1163 (1920) ; Zoller, *ibid.*, **13,** 510 (1921) ; Trimble and Bell, *Creamery Milk Plant Monthly*, **19,** (8), 40 (1930).

The skim milk, fresh and as free from fat as possible, is placed in the precipitating vat at a temperature not exceeding 35° C. Dilute HCl (1 HCl 20° Bé to 8 water) is added to the milk in a fine spray with constant stirring until the milk "breaks." The curd is permitted to settle and half the whey drained off through cloth. The curd and residual whey are stirred and more acid is added until the pH of the whey is at 4·8—4·6, when drainage is carried to completion. The curd is now washed twice with water *at a pH of* 4·7, and then is filled into a press in thin layers, yielding a final pressed cake of about 2·5 inches thickness. This cake is ground in a curd mill, spread in $\frac{1}{4}$ inch layers on trays in a drying tunnel, and dried by a strong air current at 50° C. The commercial product analysed 7·37—8·73 per cent. moisture, 1·9—2·9 per cent. ash, and 0·08—0·24 per cent. fat.

Zoller extended the investigation on grain-curd casein, finding that the casein obtained from fresh skim milk was quite different to that given by pasteurised skim milk, other conditions being the same. The curd was softer and the grains were not so definite in shape as well as being smaller, their handling being uneconomical in practice. The remarkable fact was established that in the washing of the grains with water at pH 4·7 there was a pronounced retrogression of the hydrogen ion concentration.

Fresh skim milk was heated to various temperatures, and N.HCl added to an end-point reaction of pH 4·7. The curd was well-drained and washed with water (pH 4·6) and within a few minutes the pH of the supernatant water of this suspension had increased to 5·6. This retrogression was noticed even after ten washings, at which stage the casein began to disperse rapidly in the water, an experience contrary to that with normal grain-curd casein. The decrease in acidity is believed to arise from the marked precipitation of alkaline earth phosphates during pasteurisation, which are subsequently redissolved in the acid wash water. The higher the pasteurising temperature, the more is this retrogression held in check, since the precipitated phosphates become retained in the curd as it gels, thus reducing the opportunity for redissolving in the acidified water.

Zoller's work demonstrated that "the curd at the isoelectric point is in an extremely sensitive condition and responds in a maximum degree to physical stimuli." Not only the degree of heating the milk but the duration of such heating is of importance, probably because of denaturation changes in the milk proteins, which then possess an abnormal affinity for water. Thus, treating skim milk as in the grain-curd method, draining the curd so far as possible, washing once by decantation and then draining the curd for 30 minutes, the following results were noted :—

Skim Milk.	Temperature of Preheating.	Duration of Preheating.	Temperature of Precipitation.	Moisture Content of Curd.
A	50° C.	60 mins.	34° C.	44·6 per cent.
B	63° C.	60 ,,	34° C.	62·2 ,,
C	75° C.	60 ,,	34° C.	68·5 ,,
D	100° C.	30 ,,	34° C.	79·4 ,,
E	120° C.	15 ,,	34° C.	88·2 ,,
F	Control	*Nil*	34° C.	40·3 ,,

The higher the temperature of heating the milk, the greater was the water content of the curd, this at excessive temperatures approaching a gel. Indeed, the milk heated in a sealed bomb to 140° C. gave a curd gel. Similarly, Zolier [1] reports that solutions of pure casein in carbonate-free NaOH and KOH when heated to 118° —135° C. in sealed tubes, throw down curd to an extent depending on the temperature and the duration of heating. Those caseinate solutions whose initial pH reactions were $<6{\cdot}5$, remained clear even after 40 minutes at 135° C. During the regular heating period the acidity increased by 0·18—0·54 pH. The curd thrown down was soluble in acids and alkalies and was designated as β-casein.

Apparently the power of curd to retain water is a reversible process, since precipitation of the curd in a medium heated to the original pasteurising temperature results in a firm curd again, "although its internal structure is still abnormal."

When the skim milk was pasteurised for 1 hour at 63° C. and the acid precipitation effected at different temperatures, it was observed that a very firm-grained and easily-washed curd resulted when the temperature of precipitation was 42·5° C. This is in contrast to the narrow working temperature zone of 34° — 35° C. fixing the optimum precipitation of grain-curd casein from normal skim milk.

Pasteurising at constant temperature (63° C.) it was found that the optimum temperature for acid precipitation *increased with the duration of pasteurisation*, ranging from 40° C. for 20 minutes heating to 50° C., for 100 minutes heating. Likewise, maintaining a constant pasteurising time of 1 hour, the optimum temperature of acid precipitation rose as the pasteurising temperature was raised. For pasteurising temperatures of 55° C., 80° C., and 100° C., the temperatures for precipitation were 42·5° C., 50° C., and 52·5° C. respectively.

Rennet Casein. This type of casein finds application in the manufacture of casein plastics. Only fresh skim milk is set with rennet at 35° C. for about 15—20 minutes. The curd is then cut and the temperature raised to 65° C. After about 10 minutes settling the curd is drained of whey and washed with water at 25° C., such

[1] *Science*, **52**, 614 (1920).

washing being repeated twice again at 26°—32° C. The drained curd is then heavily pressed for 60 minutes, ground and dried at 43°—46° C. The product is characterised by an unusually high ash (in the region of 8 per cent.).

Pasteurised skim milk (65° C. for 60 minutes), adjusted to pH 6·2 with HCl, was coagulated with rennet at 37° C. Zoller found that the curd had to be digested in the whey at 60° C. for half an hour in order to expel water and salts. The ash content of the final product was 5·8 per cent., due to the enmeshing in the curd of insoluble $CaHPO_4$ and $MgH\ PO_4$.

Pure Low-Ash Casein. Van Slyke and Bosworth have described a method which permits the preparation in 10 hours of a casein free from inorganic P and Ca, the product readily dissolving in dilute mono-basic alkali solutions or in excess of lime water. The aim is to approximate the natural souring of milk, since milk when just coagulated by natural souring contains all its Ca and inorganic P in solution. These workers add normal lactic acid very gradually below the surface of the skim milk, effecting immediate distribution by means of high-speed stirring. Thus there is no coagulation at the point of entry of the acid in the milk, to each litre of which about 90 c.c. of acid are added. Over 3 hours are occupied in this acidification stage. When coagulation is complete (as checked by a centrifugal test) the mixture stands for 2—4 hours, gently stirred.

The precipitated casein is repeatedly washed with water in a centrifuge until incipient re-dispersion manifests itself. It is then suspended in water and 0·1 N . NaOH added to bring the reaction to pH 7·0. Centrifuging now removes the major portion of Ca and Mg phosphates from suspension. The sodium caseinate is now diluted to a 1—1·5 per cent. concentration (as casein) and N. acetic acid added until the pH is 4·7. The casein is recovered in a centrifuge and then suspended in water, which is adjusted by addition of acetic acid to pH 4·7. Electrolysis of this suspension removes the final trace of phosphates, the suspension being placed in the centre compartment of a three-compartment cell separated by collodion sheets.

The electrolysed casein suspension is centrifuged and the solid washed with hot neutral 70—80 per cent. alcohol, followed by absolute alcohol, absolute ether and then toluene. The ash of the pure casein varies from 0·05 to 0·15 per cent.

Northrop [1] has pointed out the interesting fact that when casein is precipitated at its isoelectric point by acid, some of the casein is " denatured " and remains permanently insoluble. He extended Van Slyke and Bosworth's method of preparation, at first precipitating casein in the manner described by them. The precipitate

[1] *J. Gen. Physiol.*, **5**, 749 (1928).

is then thoroughly washed, suspended in water and the medium brought to pH 2·5—3·0 with HCl. The solution is filtered clear and the pH value is again adjusted, this time to pH 4·7, using NaOH. The precipitated casein is washed with water and dried with ether, and the resulting product gives a practically water-clear solution in alkali or acid.

Recently, Lebermann [1] has emphasised that the buffering effect of cow's milk is so pronounced that it must be taken into account in the isoelectric precipitation of casein. He recommends the precipitation of casein from skim milk by means of an acid + acetate mixture adjusted to pH 3·74.

Lactalbumin. This protein is present in milk to the extent of about 0·5 per cent. and there is evidence of its probable identity with blood serum globulin.[2] According to Wiegner [3] the degree of dispersion of lactalbumin in milk varies from 5—15 μ diameter.

To separate lactalbumin from milk, the latter is saturated with $MgSO_4$, filtered, and the filtrate rendered turbid with the addition of 0·025 per cent. solution of acetic acid. Later, the precipitate is filtered off and then re-dissolved. The solution is neutralised and the precipitation with $MgSO_4$ and acetic acid is repeated several times. Removal of electrolyte by dialysis, followed by precipitation with alcohol gives a white powder, which is washed with ether and dried *in vacuo*. An amorphous, tasteless powder results, which is soluble in saturated aqueous $MgSO_4$, but insoluble in saturated $(NH_4)_2SO_4$ or alcohol. It is precipitated from solution by addition of tannin or phosphotungstic acid.

Rupp [4] investigated the effect of heating solutions of lactalbumin. After 30 minutes at 71° C., 30·8 per cent. was coagulated, maximum coagulation occurring at pH 4·5 and giving a flocculent precipitate quite unlike the well-known coagulum resulting when egg albumin is heated. The isoelectric point [5] is at pH 4·55.

Lactoglobulin. Sebelin [6] observed this protein in whey. It occurs in milk to the extent of about 0·03 per cent. Osborne and Wakeman [7] were able to prepare 0·52 gr. dry, ash-free globulin per litre of milk. They report that it may be a lecithalbumin or a mixture of proteins, and its solubility reactions have little in common with most other globulins.

For its isolation, milk is saturated with NaCl, the precipitated casein filtered off, the filtrate warmed to 35° C., filtered again and

[1] *Biochem. Z.*, **206,** 56 (1929).
[2] Crowther and Raistrick, *Biochem. J.*, **10,** 434 (1916); Hartley, *ibid.*, **8,** 541 (1916).
[3] *Z. Untersuch. Lebensm.*, **27,** 425 (1914).
[4] U.S. Dept. Agr., *Dept. Bull.*, **166** (1913). Cf. Freudenreich, *Rev. gén. Lait.*, **4,** 433 (1925).
[5] Csonka *et al.*, *J. Am. Chem. Soc.*, **48,** 763 (1926).
[6] *Z. physiol. Chem.*, **9,** 445 (1885).
[7] *J. Biol. Chem.*, **33,** 7 (1918).

then saturated with $MgSO_4$. The globulin is now filtered off and may be purified by dissolving in saturated brine and repeating the above operations.

Lactoglobulin is insoluble in water and is amphoteric in character, the acidic function being more pronounced than the basic. Its solutions are completely coagulated by heating to 72° C.

COLOSTRUM

Immediately after calving, the cow yields a rich milk characterised by high protein content, mainly globulin. Just what is the true function of colostrum, bovine or human, is not quite agreed upon, but the view gains favour that the globulin content is connected with certain immunising properties.[1] The composition of colostrum alters rapidly with time, as shown in the following analytical table by Leach [2] :—

TABLE 40. COMPOSITION OF COLOSTRUM AS A FUNCTION OF TIME.

Time after Calving.	Casein per cent.	Globulin per cent.	Fat per cent.	Lactose per cent.	Ash per cent.	Total Solids per cent.
At once .	2·65	16·56	3·54	3·00	1·18	26·93
10 hrs. .	4·28	9·32	4·66	1·42	1·55	21·23
24 hrs. .	4·50	6·25	4·75	2·85	1·02	19·37
48 hrs. .	3·25	2·31	4·21	3·46	0·96	14·19
72 hrs. .	3·33	1·03	4·08	4·10	0·82	13·56

Recently Provan [3] has published interesting data relating to the colostrum of Shorthorn cows, comparing the second and third milkings (12 and 24 hours after calving respectively) with the average composition of 185 samples of normal milk from cows in the same herd ; see Table 41, where the figures give per cent. values.

As pointed out by Mai and Keller,[4] colostrum can be readily differentiated from cow's milk owing to its relatively high content of chlorine with low content of lactose. Colostrum coagulates on being heated for 15 minutes at 70° C., but this characteristic disappears about 72 hours post partum.[5]

Hill,[6] in his investigation of the toughness of the curd produced by coagulating milk (see p. 279) has observed that colostrum gives

[1] Paterson and Smith, " Modern Methods of Feeding in Infancy and Childhood " (London, 1929), p. 23.

[2] " Food Inspection and Analysis " (New York, 1907), p. 93. Very detailed analytical and physico-chemical data have been given by Engel and Schlag, *Milchwirtschaft. Forsch.*, **2,** 1 (1924) and Mrozek and Schlag, *ibid.*, **4,** 183 (1927). For analyses of human colostrum see Hammett, *J. Biol. Chem.*, **29,** 381 (1917) ; Lowenfeld, *Biochem. J.*, **21,** 1 (1927).

[3] *Welsh J. Agr.*, **4,** 141 (1928).

[4] *Klin. Wochschr.*, **9,** 535 (1930).

[5] Cf. Hollen, *Deut. tierärztl.*, **33,** 386 (1925).

[6] Utah Agr. Expt. Sta., *Bull.*, **207,** 9 (1928).

TABLE 41. COMPOSITION OF COLOSTRUM AND NORMAL MILK.

Analysis.	2nd Milking.	3rd Milking.	Normal Milking.
Total solids . . .	20·65	16·68	12·63
Fat	3·31	3·50	3·69
Solids-not-fat . .	18·26	13·18	8·94
Protein . . .	13·05	8·15	3·41
Ash	0·937	0·817	0·708
CaO	0·2011	0·1864	0·1646
P_2O_5 . . .	0·3390	0·3269	0·2324
K_2O	0·1902	0·2005	0·1952
Cl	0·1323	0·1135	0·0962

a tougher curd than does the normal milk. "The increase in hardness of the curd at this time is greater than the increase in the composition of proteins. This would indicate that some other factor is partially responsible. From 4—6 weeks after freshening, the curd becomes normal in texture and varies only slightly, until toward the end of the lactation period. It then may become harder than normal," although an abnormal milk may now yield a softer curd or even refuse to coagulate.

CREAM

Cream is formed by the rising of the fat globules through the denser milk serum to the surface. The density of the liquefied fat is about 0·92, whilst that of the milk serum is over unity. Thick cream contains about 56 per cent. fat and 39 per cent. water, whilst thin cream contains about 29 per cent. fat and 64 per cent. water. The fat content varies between very wide limits, and the percentage of fat is in inverse ratio to the density of the cream. According to Stokes' law, for a constant medium, the velocity of ascension [1] of the globules varies inversely as the square of their radius. The Stokes' equation is :—

$$V = \frac{2r^2 (d - d^1) g}{9\eta}.$$

Where V = constant velocity of dispersed phase (globules).
r = radius of the globules.
d = density of the globule phase (*i.e.*, fat).
d^1 = density of the medium (milk serum).
η = viscosity of the medium.
g = the gravity constant.

Van Dam and Sirks,[2] and van der Burg [3] have checked up the rate

[1] Cf. Groschuff, *Kolloid-Z.*, **9**, 259 (1911).
[2] *Verslag. Land. Onderzoek. Rijkslandbouwproefsta.*, **26**, 106 (1922).
[3] *Forsch. Geb. Milchw. Molkereiwesens*, **1**, 154 (1921).

of ascension of fat globules in milk and found good agreement with values calculated from Stokes' law.

The usual view of the creaming of milk will be gathered from the two following quotations from leading authorities : " This separation of fat in the form of cream is possible because of the difference in S.G. between the fat globules and the other constituents of the milk. If the milk is allowed to stand undisturbed, the fat particles will slowly rise to the surface, and can then be skimmed off in the form of cream, the rapidity and completeness with which the fat globules rise to the surface depending on the difference in specific gravity between the fat globules and the other elements in the milk, and on the viscosity of the milk serum." (Stocking.) [1]

" The principal agent retarding or preventing the upward passage of the fat globules is the viscosity of the milk, which is largely due to its . . . constituents of a colloidal nature inherent in milk and cream, and to the milk sugar. If the viscosity of the milk were no greater than that of water, the fat globules would rise to the surface instantaneously in a similar manner as oil poured into water rises to the surface." (Hunziker.)[2]

Recent work by Rahn has shown the usual view of creaming as given above to be incomplete, and, as regards the viscosity factor alone, quite untenable.

Ordinary gravity creaming of milk may be carried out in three ways :—

(i.) Standing the milk in shallow pans to a depth of 2 to 4 inches for 24 to 36 hours. The cream is removed by a skimmer, but from 0·5 to 1 per cent. of the cream is left in the skim milk.

(ii.) Standing the milk in deep pans (about 20 inches) set in cold water. The skim milk is drawn from the bottom and the amount of fat retained in the skim milk is only about 0·2 per cent. Bancroft [3] has pointed out that a more rapid rise of cream occurs in a deep pan than in a shallow one. The finer globules are filtered out by the ascension of the larger. Woodman [4] proved experimentally that emulsions creamed more rapidly the wider the vessel used.

(iii.) The water dilution method ; to the new milk is added hot or cold water to the amount of $\frac{1}{4}$ to $\frac{1}{3}$ by volume. The idea involved is that lowering the viscosity of the milk serum will accelerate the rising of the fat globules. The results, however, are not satisfactory,[5] and about 0·7 per cent. of fat is retained in the skim milk.

Rahn [6] has shown that the viscosity of milk can be increased, yet instead of the creaming being slowed down, it is accelerated.

[1] " Manual of Milk Products " (New York, 1917), p. 225.
[2] " The Butter Industry " (Illinois, 1920), p. 68.
[3] Cf. Bancroft, " Applied Colloid Chemistry " (New York, 1921), p. 193.
[4] *J. Pomology Hort. Sci.*, **4**, 190 (1925).
[5] See Stocking, *loc. cit.*, 228, where the work of Wing on the water dilution method is discussed.
[6] *Forsch. Geb. Milchw. Molkereiwesens*, **1**, 133 (1921).

Moreover, the increase in viscosity is attained by adding water-soluble colloids, *e.g.*, gelatin. Using warm milk at 40° C. containing gelatin, Rahn reports : " The addition of gelatin caused a quicker cream-rise, a thicker cream-layer, a looser cream of small fat content, a much more complete separation of fat as cream and also a skim milk of lower fat content, in spite of the fact that the viscosity much exceeded the highest value for normal milk which lies about 2·4." Other colloids gave similar results and even when the viscosity was increased so high as 5·61 there was a much better yield of cream than with the untreated milk.

Other colloids, such as gum tragacanth, gum arabic, peptone and albumin, in small quantities, accelerated creaming. Increasing the viscosity of the milk by adding non-colloids like sugar, delayed creaming. Obviously these results demand a new interpretation of the phenomenon of creaming.[1]

Rahn further showed that the inhibited creaming of heated milk (formerly explained as due to the loading of the fat globules by coagulated protein—lactalbumin) could be changed to a normal value by adding gelatin or other accelerating colloid *after* heating. By measuring the velocity of ascent of fat globules in milk under various conditions, Rahn proved that in ordinary milk the fat globules do not rise individually, but clump together, such clumps possessing greater buoyancy. Raw milk contains numbers of these clumps, but heated milk only contains few, and herein lies the essential difference in the mode of creaming. Heating was found to destroy clumping of the fat globules.[2] The addition of albumin, gelatin, etc., enhanced clumping due to the formation of an adsorbed colloidal envelope " which permits the sticking-together of the globules as they collide owing to their molecular movements." Analytical proof is given of this concentration around the fat globules in the case of gelatin, nitrogen determination being carried out in the cream and skim milk. The following data by Rahn show that the added colloid resulted in a looser cream of diminished fat content :—

FAT CONTENT OF CREAM.

	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Gelatin added . . .	*Nil*	0·35	0·70	1·05	1·40
Fat content . . .	33	30	27	25	23
Gum arabic added . .	*Nil*	1·18	2·36	—	—
Fat content . . .	27	26	25	—	—

[1] Rahn, *loc. cit.*, Sakurei, *Biochem. Z.*, **149,** 525 (1924) ; Palmer and Anderson. Minnesota Agr. Expt. Sta., *Ann. Rept.* (1922), p. 39 ; Traube, Brit. Pat. 226,440 (1924) ; Van Dam and Sirks, *Verslag. Land. Onderzoek. Rijkslandbouwproefsta.*, **26,** 106 (1922) ; Hekma and Sirks , *ibid.*, **29,** 94 (1924) ; Sirks, *ibid.*, **29,** 137 (1924).

[2] Cf. Weinlig, *Forsch. Geb. Milchwirtsch. Molkereiwes.*, **2,** 127 (1922).

Heating reduces the adhesiveness of the envelope-colloid and the fat globules rise singly, a thicker cream layer being the result. In this connection the following table given by Rahn [1] is of interest :—

TABLE 42. EFFECT OF GELATIN ON THE CREAMING OF RAW AND HEATED MILK.

Time Elapsed.	Raw Milk.		Milk heated 10 mins. at 100° C.	
	No Gelatin.	1 per cent. Gelatin.	No Gelatin.	1 per cent. Gelatin.
0·5 hour .	3 mm.	8 mm.	*Nil.*	*Nil.*
1·0 ,, .	9·5 ,,	16 ,,	,,	1 mm.
2·5 hours .	11 ,,	19 ,,	,,	3·5 ,,
4·0 ,, .	13 ,,	21 ,,	2 mm.	5 ,,
18·0 ,, .	16 ,,	28 ,,	3 ,,	16 ,,
48·0 ,, .	18 ,,	29 ,,	5 ,,	21 ,,
Fat in cream . .	25·8 per cent.	22·5 per cent.	31·1 per cent.	31·2 per cent.
Fat in skim milk .	1·1 ,,	0·3 ,,	2·2 ,,	0·2 ,,
Actual fat in cream .	4·50 gr.	6·50 gr.	1·65 gr.	6·55 gr.
Actual fat in skim milk	2·56 ,,	0·66 ,,	5·39 ,,	0·46 ,,
Degree of creaming .	65 per cent.	91 per cent.	24 per cent.	93 per cent.

Van Dam and Sirks [2] also found that such colloids as gum tragacanth, gelatin, agar, starch, Irish moss, etc., added to milk, resulted in a 15—25 per cent. increase in the cream volume on setting the milk at 9° C., although the viscosity increased about 25 per cent. Clumping of the fat globules was postulated. They found no relation between creaming ability and the CaO and H_3PO_4 content of milk. Addition of acid and alkali retarded creaming in the case of fresh milk.

Palmer and Anderson [3] agree that the plasma colloids are more important than the fat globules in relation to the creaming of milks with a uniform fat content. Making a special study of the effect of pasteurisation [4] on creaming, they demonstrated that (*a*) calcium caseinate hinders satisfactory creaming, (*b*) the whey colloids are effective promoters of cream rising, a statement confirmed later by Troy and Sharp,[5] (*c*) pasteurisation increases the hindering action of calcium caseinate. The general conclusion reached is " that both exhaustiveness of rise of fat and greater volume of cream are promoted by the more truly hydrophilic colloids and depressed by colloids with hydrophobic properties. Pasteurisation at 63° C. for 30 minutes increases the effectiveness of the former but decreases the effectiveness of the latter."

[1] " Physik der Milchwirtschaft " (Berlin, 1928), p. 58.
[2] *Loc. cit.*, **26,** 106 (1922).
[3] *J. Dairy Sci.*, **9,** 1 (1926).
[4] Palmer *et al.*, *ibid.*, **9,** 171 (1926).
[5] *Ibid.*, **9,** 216 (1926).

The authors do not agree with Rahn that the explanation of cream rising is to be based on the clumping of fat globules. They emphatically state that this is not the main factor involved. However, no alternative theory is offered, and other workers have since supported Rahn's conclusions with valuable experimental evidence.

Troy and Sharp [1] have published a lengthy paper, accompanied by interesting photomicrographs, strongly supporting the theory that clumping of fat globules underlies creaming. Using known physical constants of milk, a table was prepared showing the rate of rise of *individual* fat globules of various sizes as calculated from Stokes' law. Calculated values show that *contrary to practical experience*, creaming should occur more quickly at 25° C. than at 5° C., *i.e.*, in practice, the globules do not rise singly. Actual experimental determination of the rate of rise of single fat globules agreed well with the rate as calculated from Stokes' law. Thus, the logarithms of the calculated rate of rise when plotted against the logarithms of the diameters of the globules, give a straight line graph.

Troy and Sharp next measured the rate of ascent in milk plasma of clumps of fat globules (varying in size from 10 μ to 800 μ), and again the results agreed with those calculated from Stokes' law. The rate of ascent is sufficient to account for the practical experience of creaming. The authors point out that the experimental vision of the clumps is difficult because of their fragile character. Some clumps contain only a few globules, others contain thousands. Agreeing with Rahn and Van Dam and Sirks, Troy and Sharp believe that the clumps contain milk plasma, and the densest spherical clumps probably contain slightly less than 50 per cent. by volume of milk fat. The plasma between is relatively fat free. Deep cream layers contain large irregular stable clumps, whilst "compact approximately spherical clusters, and especially weak clusters, form shallow layers and the fat content of the cream is high." Setting the cream at higher temperatures weakens the clumping effect and permits closer packing. A deeper cream layer therefore results at lower creaming temperatures.

An important technical consideration of the influence of heat on creaming concerns the pasteurisation of milk. An exhaustive study of this subject has been made by Whittaker and his co-workers,[2] who find that pasteurisation : (1) at 61° C. for 30 minutes resulted in a practically unchanged cream volume, or even a slight increase ; (2) at 63° C. for 30 minutes, the cream volume was reduced over a range of about 8 per cent. This was more marked with fresh milk, due to its initial greater cream volume, than with old milk. A good cream volume is favoured by the absence of agitation during the

[1] *Ibid.*, **11,** 189 (1928).
[2] U.S. Dept. Agr., *Dept. Bull.*, **1344** (1925).

holding period, and by cooling to below 7° C. after pasteurisation. Burri [1] agrees that unheated milk creams more rapidly than milk pasteurised above 61° C. Boiling the milk very seriously reduced creaming.[2]

Much remains to be worked out concerning the fundamental physico-chemical factors concerned in cream as an emulsion. The peculiar effect of added colloids, as well as the thermal change operating above 61° C. require investigation. The nature and extent of the adsorption of milk colloids and added colloids at the fat-serum boundary need to be worked out. Preferential or mixed adsorption of the colloids is indicated, as well as a study of the thickness and character of the adsorbed films round the fat globules.

Centrifugal Separation of Cream. Separation of an emulsion of fat in aqueous medium depends on the difference in specific gravity of the two phases. Centrifugal separation [3] is an enhanced gravity settling, the gravitational force being enormously magnified. The fat globules move through the aqueous phase with a velocity influenced by such factors as (1) centrifugal force exerted; (2) viscosity of the aqueous medium; (3) difference in the density of the phases; (4) relation between the mass and the surface area of the globules; (5) temperature.

From Stokes' equation it is obvious that although the specific gravity difference is important, such importance rapidly diminishes when the diameter of the globule is sufficiently reduced, since the velocity is proportional to the square of the radius.

The separation of cream may be effected in many types of centrifugal separators, and the following discussion will be based on the use of the modern de Laval type.

The essential part of the machine is a bowl, capable of being revolved at high speed, containing a series of round, sloping, conical steel discs, superimposed and spaced slightly apart by thin calks or ribs fastened to them. Milk is fed into the centre of the bowl by a distributing device contained in the central shaft, and passes through tangential slots in the wall of the shaft into enclosed gutters. It now flows from the lower ends of these gutters (which extend beyond the cream wall), and is delivered into the neutral zone or separating disc space of the bowl, passing upward into the various discs through a series of openings in the discs located above and in line with the distributing device outlets. The important result is thus obtained of delivering the incoming milk into the separating discs without interference of the cream wall or mixing of the incoming milk with the already separated cream. The separation then takes place in the

[1] *Milchw. Zentr.*, **45,** 33 (1916).

[2] See table of data by Rahn and Sharp, " Physik der Milchwirtschaft " (Berlin, 1928), p. 53.

[3] Ayres, *Ind. Eng. Chem.*, **13,** 1011 (1921); Mork, *Meldinger Norges Landbruks*, **7,** 1-60 (1927).

discs, the cream particles moving inward toward the centre and forming the cream wall round the central shaft and distributing device and mounting upward to the point of discharge through the cream outlet. The skim milk moves outward beyond the discs and is carried upward along the wall of the bowl to the points of discharge through the skim milk outlets (see Fig. 24).

It can be shown [1] that under the influence of centrifugal force, the time required for a globule of radius r to travel through a given

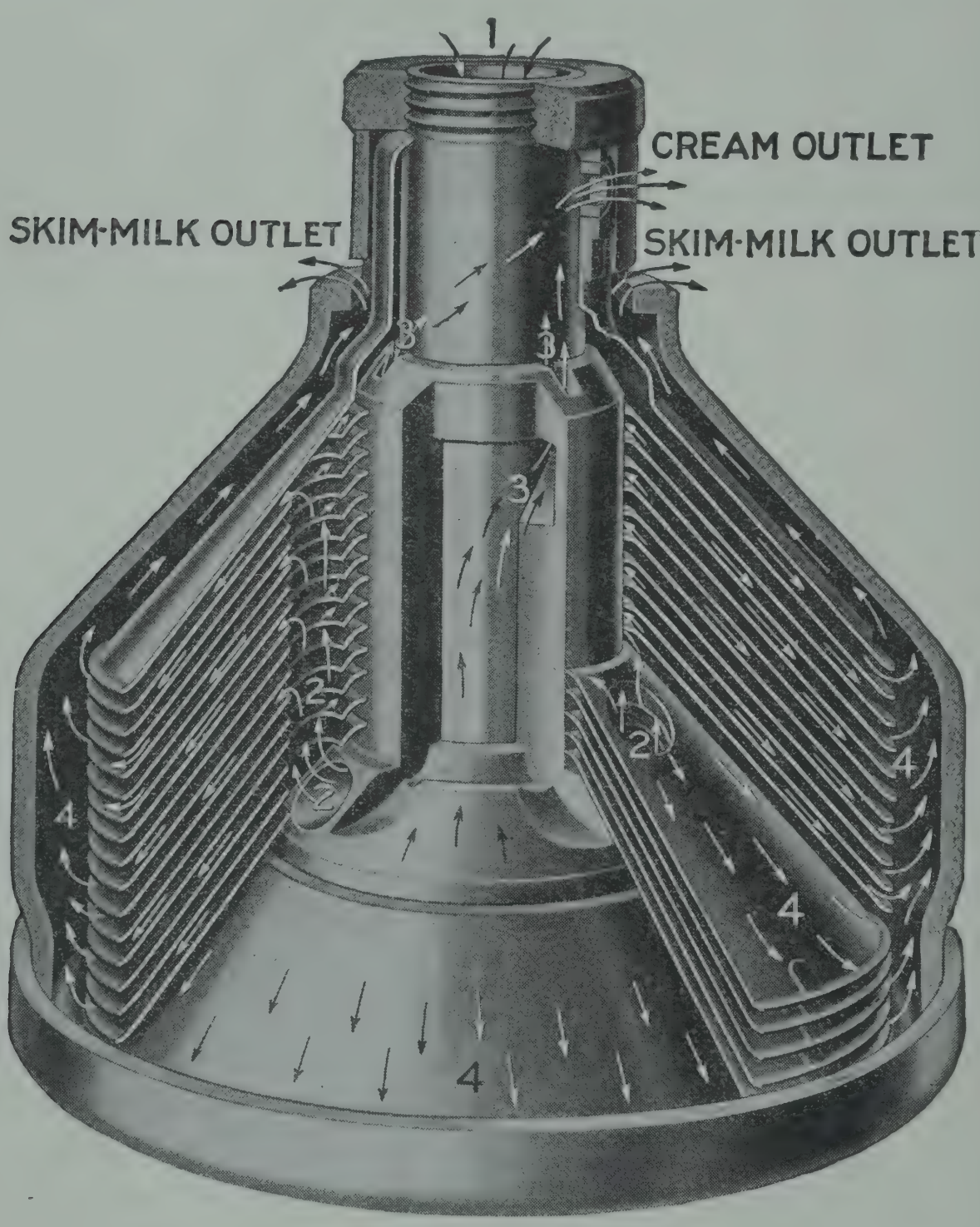


FIG. 24.—Cross-section of de Laval cream separator.

space is proportional to the square root of the cube of the radius, *i.e.*, $r^{3/2}$. As the number of gallons of milk handled per hour by the machine is inversely proportional to the time, it follows that for any given radius of globule there is a critical value where its velocity against the stream of milk will be equal to the velocity of the stream itself, so that globules of smaller radius pass out with the stream of separated milk. The fat content of the separated milk is proportional to the cube root of the square of the number of gallons per hour.

[1] Richmond, "Dairy Chemistry" (London, 1914), 255—9; Sharp and Troy, *J. Dairy Sci.*, **11,** 259 (1928).

Centrifugal machines for cream separation are usually run at 6,000 revolutions per minute.[1] The intensity of the centrifugal force exerted is directly proportional to the mass of the contents of the bowl, to the diameter of the bowl, and to the square of the speed of revolution.[2] The ratio of cream to separated milk will depend on the relative distances of their respective outlets from the centre of the bowl under normal running conditions.

An important figure in cream separation is the capacity of the separator, *i.e.*, the quantity of milk which can be separated per hour. Obviously, this depends on the centrifugal force and on the rate of inflow of the milk. Any increase over the optimum capacity will lower the efficiency of the separator, since the milk will necessarily be subjected to centrifugal action for a shorter period ; this means a loss of butterfat, carried away in the separated milk. The rate of inflow of the milk requires careful control ; reduction of the rate below the normal value does not lead to increased efficiency of separation, merely lengthening the time required.

Cream is usually separated at 29°—35° C. At lower temperatures the viscosity of the milk increases and the fat globules are influenced by the greater resistance offered to the centrifugal separation. Though the cream is richer, due to the slower passage of the milk through the machine, the loss is greater, since the separated milk contains more than the usual amount of fat.

The quality of the milk is important. The richer it is in butterfat, the richer is the cream and the greater the loss in the skim milk. As an approximate guide, the percentage of butterfat in the cream is proportional to the fat content of the whole milk.

Sharp [3] has made an interesting study of the physical constants of milk as influencing the centrifugal separation of cream at different temperatures. He shows that the rate of movement of a fat globule through the milk plasma due to centrifugal force at any instant is given by the equation :—

$$V = \frac{2r^2(d_p - d_f)4\pi^2n^2R}{9\eta 3600}$$

or

$$V = \frac{0{\cdot}00244(d_p - d_f)r^2n^2R}{\eta}$$

where r = radius of the globule.
η = viscosity of the milk plasma.
d_p = density of the milk plasma.
d_f = density of the fat.
n = number of revolutions of separator bowl per minute.
R = distance of the fat globule from the axis of rotation.

[1] *Vide* Hunziker, " The Butter Industry " (1927), 77—97.
[2] *Milchwirtschaft. Forsch.*, **2**, 382 (1925).
[3] *J. Dairy Sci.*, **11**, 259 (1928).

If K be a constant covering all the factors in the equations, which are independent of the dimensions and speed of the separator and the size of the globules,

$$K = \frac{0{\cdot}00244(d_p - d_f)}{\eta}$$

Under the constant operating conditions, the effective force tending to the separation of the fat globules depends on the magnitude of K, and would thus be more effective the greater the difference in density of the phases and the lower the viscosity. Both factors are influenced by temperature, and the following table by Sharp illustrates this.

TABLE 43. DENSITY OF PLASMA AND FAT, AND THE VISCOSITY OF THE PLASMA AS AFFECTING THE VELOCITY OF MOVEMENT OF THE FAT GLOBULES UNDER CENTRIFUGAL FORCE.

Temp.	d_p	d_f	$d_p - d_f$	η	K	K^1	Percentage Increase in K^1 per 5° C. Rise in Temp.
0° C.				Poise.			Per cent.
5	1·0365	0·9612	0·0753	0·0296	0·0062	0·0062	—
10	1·0359	0·9528	0·0831	0·0247	0·0082	0·0083	33·0
15	1·0348	0·9421	0·0927	0·0210	0·0108	0·0110	33·0
20	1·0338	0·9304	0·1034	0·0179	0·0141	0·0144	30·9
25	1·0322	0·9208	0·1114	0·0154	0·0176	0·0181	25·9
30	1·0306	0·9119	0·1187	0·0133	0·0218	0·0226	24·9
35	1·0288	0·9082	0·1206	0·0117	0·0252	0·0262	15·9
40	1·0266	0·9050	0·1216	0·0104	0·0285	0·0297	13·4
45	1·0245	0·9012	0·1233	0·0093	0·0323	0·0337	11·3
50	1·0223	0·8982	0·1241	0·0085	0·0356	0·0372	10·4
55	1·0198	0·8945	0·1253	0·0077	0·0396	0·0415	11·4
60	1·0171	0·8913	0·1258	0·0071	0·0432	0·0454	9·1
65	1·0145	0·8881	0·1264	0·0066	0·0467	0·0492	8·4
70	1·0117	0·8848	0·1269	0·0062	0·0499	0·0527	7·1
75	1·0086	0·8813	0·1273	0·0059	0·0526	0·0558	5·9
80	1·0054	0·8778	0·1276	0·0057	0·0546	0·0580	3·9

The most marked difference in phase densities occurs between 5° C. and 35° C., the increase being rapid, whilst the viscosity decreases somewhat rapidly up to about 40° C. Thus K increases rather rapidly with rise in temperature up to the range 35° C. to 40° C.; it is not so pronounced thereafter. "Still another factor which tends to make the efficiency of separation increase more rapidly with temperature up to 35°—40° C. is the actual expansion of the fat globules. If the size of the fat globules is measured at 5° C. their volume will be 6·2 per cent. greater at 40° C., while they will increase in volume only about 3 per cent. in going from 40°—80° C. It was assumed that the fat globules were measured at 5° C. and the effect of the increase in size of the fat globules with the increase in temperature as influencing K is given as K^1."

SKIM MILK

The milk remaining after cream separation is termed skim milk and contains about 0·15 per cent. of fat, whilst the protein, lactose and ash analysis are similar to those of whole milk.

The size-frequency analysis of the fat globules has been determined by Rahn [1] and his results are given in Table 44 :—

TABLE 44. SIZE-FREQUENCY ANALYSIS OF FAT GLOBULES IN SKIM MILK.

Diameter of Globules in μ.	Comparative Number of Globules in Unit Volume.	Percentage of the Total Fat Present in Unit Volume
0—1	41·8	1·7
1—2	47·7	40·4
2—3	9·2	30·8
3—4	0·9	13·4
4—5	0·3	8·0
5—6	0·1	6·0
6	*Nil.*	*Nil.*

MILK-FROTH

New milk readily froths, *e.g.*, after just drawing, on stirring or pumping, and on passage through a cream separator.[2] Normal milk contains about 10 per cent. by volume of CO_2.[3] Aeration reduces the CO_2 content and increases the oxygen and nitrogen contents, particularly the latter. Milk foam is a technical nuisance, and foam destroyers are frequently employed in dairies. One excellent method is to run the milk into a detention tank, drawing the milk continually from the bottom. Thus a round tank, 16 inches in diameter and 36 inches high, permitting a foot head of froth, has been used to eliminate the froth from violently-agitated milk at the rate of 400 gallons per hour, the time of detention being approximately $3\frac{1}{4}$ minutes.[4]

Foam on milk is a disadvantage during pasteurisation. Thus, in the "Holding Method," where the milk is held at 145° F. for 30 minutes, the froth on the surface of the milk may be air-cooled and undesirable bacteria not destroyed. The foam in commercial pasteurisers may be from 8°—10° F. below the main bulk of the milk.[5] The most recent plants either heat the air in the holder

[1] See "Physik der Milchwirtschaft" (1928), Chap. 4.
[2] *Ibid.*, p. 17.
[3] Van Slyke and Baker, *J. Biol. Chem.*, **40,** 335 (1919); Marshall, Mich. State Agr. Expt. Sta., *Special Bull.*, **16** (1902); Kohman, *Ind. Eng. Chem.*, **15,** 273 (1923).
[4] *Vide* Stevenson, U.S. Public Health Reports, *Reprint*, No. **608,** 17 (1920).
[5] Smith, *J. Exptl. Med.*, **4,** 217 (1899); Whittaker, U.S. Dept. Agr., *Tech. Bull.*, **18** (1927).

with steam, or introduce such engineering modifications as prevent milk splashing.[1]

When milk is agitated in such a way that the boundary air/liquid is considerably extended, adsorption of the milk colloids at this boundary is intensified. Froth or foam represents an enormous film-like area of liquid supported in a gas (usually air). The air-bubbles persist for some time owing to the presence of the milk-colloids in a solid condition (the Ramsden phenomenon). In the case of milk-froth, quantitative proof of the Ramsden phenomenon has been given by Siedel and Hesse.[2] Examining the froth on skim milk after cream separation by centrifuging, they found an increase in the protein content but no change in the inorganic content. The following table of their results is quoted by Rahn [3] :—

TABLE 45. ANALYTICAL COMPARISON OF MILK AND MILK-FOAM.

Analysis of Dry Solids.					
Strongly Frothing Milk.			Ordinary Milk.		
	Protein. (per cent.)	Ash. (per cent.)		Protein. (per cent.)	Ash. (per cent.)
Froth . .	38·87	8·63	Froth . .	36·39	8·52
Milk . . .	35·60	8·58	Milk . .	34·72	8·57
Excess in froth .	+3·27	+0·05	Excess in froth .	+1·67	−0·05

Froth allowed to remain undisturbed in milk gradually collapses, and eventually wrinkles of delicate membrano-solid character lie on the surface. Brouwer [4] claims to have proved the existence in milk of peculiarly-shaped small bodies, which were membranes originally formed by adsorption of protein on gas bubbles and remaining in the milk after the bubbles have collapsed. Fig. 25, reproduced from Rahn,[5] shows the membranes after the collapse of milk froth from a separator.

Rahn showed that the addition of 0·5 per cent. of peptone to the milk altered the character of the milk froth, bubbles forming which burst in upon one another, gradually uniting to larger and larger bubbles. This is due to the change in character of the bubble walls, which are solid in the air phase, but redissolve in the liquid phase. Addition of gelatin to the peptone-milk causes still further change

[1] Cf. Schier, *Proc. Intern. Assocn. Milk Dealers, Plant Sec.*, p. 69 (1926); Mortensen, U.S. Pat., 1,749,257 (1930).

[2] *Molkerei-Ztg.* (*Hildesheim*), (1900), p. 638.

[3] *Kolloid-Z.*, **30,** 341 (1922). See also " Physik der Milchwirtschaft," Chap. 2, on Milchshaum.

[4] *Niederlandsch. Tijdschr. Geneeskunde*, **67,** 409 (1923); also Hekma and Brouwer, *Verslag. Land. Onderzoek. Rijkslandbouwproefsta*, **18,** 46 (1923).

[5] *Loc. cit.*, p. 6.

owing to the displacement of the peptone by the gelatin. The outer walls of the bubbles are now only semi-solid, and slow collapse of the bubbles follows the occurrence of rents in the walls.

Siedel found that churning milk at a low temperature for about 45 minutes inhibited froth formation when the milk was subsequently passed through a cream separator. Cooling the milk without churning did not affect froth formation. Also, separated milk, cooled and churned, and then mixed with cream, frothed as usual when passed through a separator. Two facts emerge from these experiments : (*a*) churning fat-poor milk renders the colloid

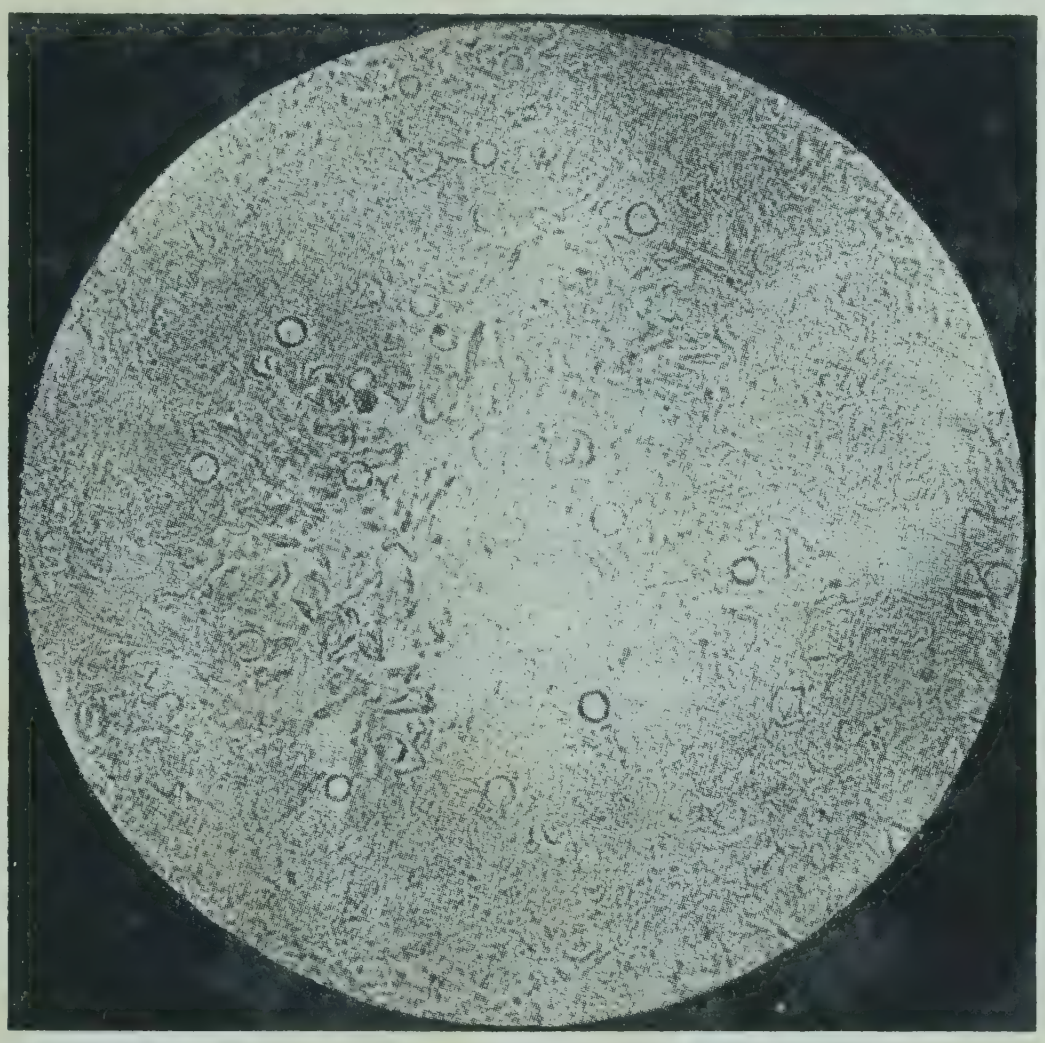


FIG. 25.—Solid foam-films from the centrifuge slime of skim milk repeatedly passed through the centrifuge.

inactive, (*b*) the greater portion of the foam colloid is in the cream. Churning skim milk causes frothing with the consequent irreversible coagulation of the milk colloids. Thus, the cause of frothing being removed, later agitation has no effect. Cream is rich in milk colloids owing to the marked adsorption occurring at the enormous fat/water interface. That the proteins of milk do concentrate round the fat globules has been proved quantitatively by Rahn [1] by analytical tests on skim milk, cream and butter.

An investigation of the phenomenon of foaming in relation to the temperature, and the surface tension of milk has been made by Mohr and Brockmann.[2] They found that there is a definite connec-

[1] *Forsch. Geb. Milchw. Molkereiwes.*, **1,** 313 (1921).
[2] *Milchwirtschaft. Forsch.*, **11,** 48 (1930).

tion between foam-solidity and surface viscosity. In general, milk gives a more stable foam the greater the tendency to form a filmed surface, and there is an optimum surface viscosity relative to foam stability. The influence of temperature is different in different samples of milk, and the optimum temperature for frothing lies within the range 30°—60° C. Leete [1] finds that the tendency of milk to foam is at a minimum between 20° and 30° C. Below 20° C. foaming increases, whilst above 30° C. the foaming tendency rises rapidly. Rahn [2] observed that agitation (constant degree) of skim milk at different temperatures shows a rapid fall in foam tendency as the temperature increases. The graph connecting foam-height with temperature shows an inflexion point, the origin of which is not clear. He reports that the foam on warm milk is quite different to that on cold milk, the latter foam remaining unaltered for a short while and then collapsing suddenly. The warm foam collapses slowly at constant velocity. Again, the foam on new milk differs considerably from that on skim milk. The former is dull-looking with large bubbles, more like dough, whilst the latter is fine-grained and stiffer, resembling whipped egg-white.

It is most probable that the skin which forms on heated milk [3] is also due to the colloids concerned in milk foam, the thin delicate skin being already present on the surface of the cold milk and becoming concentrated upon evaporation. The general subject has been investigated particularly by Ramsden.[4]

The presence in water of a solute which causes a lowering of the surface tension results in an accumulation of the solute at the air/liquid boundary (see p. 171). The accumulation may even result in an irreversible "precipitation" of the solute (the Ramsden phenomenon). In the case of milk a skin appears on the surface when vacuum evaporation is attempted at room temperature, or when normal heating above 50° C. occurs. It was formerly believed that the skin was entirely albumin, but Semlritzki [5] was able to remove successive skins to a total amount of 1·023 per cent. of the milk, *i.e.*, to an amount exceeding the albumin content. Friese [6] obtained thirty successive membranes from 1 litre of milk at 100° C. The average ash of the membranes analysed : $CaO = 49{\cdot}5$; $P_2O_5 = 43{\cdot}8$; $Fe_2O_3 = 0{\cdot}9$; $NaO + KO + MgO = 5{\cdot}7$ per cent. He also observed that the kind of container considerably affected the number of skins obtained. The time of standing affects the fat content of the milk skin. Thus in 7 hours 23 per cent. of fat is

[1] U.S. Dept. Agr., *circ.* No. **108** (1930). See also Sanman and Ruehe, *J. Dairy Sci.*, **13,** 48 (1930) ; "Fundamentals of Dairy Science" (1928), p. 172.

[2] "Physik der Milchwirtschaft," p. 16.

[3] Hertz and Jamison, *J. Physiol.*, **27,** 26 (1901).

[4] *Arch. Anat. Physiol.* (1894), 517 ; *Proc. Roy. Soc.*, **72,** 156 (1903) ; *Z. physik. Chem.*, **47,** 336 (1904).

[5] *Pogg. Ann.*, **37,** 450.

[6] *Milchwirtschaft. Forsch.*, **1,** 316 (1924).

removed, the skin analysing to : water = 48·45 ; fat = 41·43 ; protein = 4·71 ; lactose = 3·34 ; ash = 0·69 per cent. The protein was all casein with *no* albumin.[1]

" Ropiness " in milk due to surface influence has been investigated by Mattick,[2] who found " ropes " to occur on appropriate surfaces such as farm coolers, under certain favourable conditions of rate of flow, temperature and acidity. The " ropes " are of casein and/or lactalbumin and are modifications of Ramsden's " mechanical surface aggregates."

Whipped Cream. Whipped cream is a special case of milk-foam, since cream is richer than milk in fat and proteins. The remarkable stability and the great viscosity of whipped cream are due to the cell-like structure imparted by the air bubble, whose walls are stiffened not only by the denatured protein but by the more or less solidified milk fat. The latter factor vanishes on heating and the whipped cream collapses, although some foam structure persists due to the protein present.

Mohr [3] has investigated the physico-chemical factors involved, pointing out that cream of the same origin and chemical analysis may possess physical differences affecting whipping capacity according as the fat globules are solid or liquid and homogenised, coarse-grained or clustered together. He quantitatively ascertained that the duration of whipping profoundly influenced the volume of the whipped cream, the volume rising rapidly to a maximum (in about 3 minutes in his experiments) and then falling slowly to the original volume. The maximum solidity of the foam did not coincide with the maximum volume, but occurred several minutes later and then fell with extreme rapidity, butter forming with excessive beating.

Dahlberg and Hening [4] have made an extensive investigation on the viscosity, surface tension and whipping properties of milk and cream (see p. 204). It was found that a good whipping cream invariably yielded a smaller volume when whipped than did poor whipping cream. Whipping capacity was improved by ageing of the cream, increase in fat content, and increase in solids-not-fat. In a general way, factors (excluding homogenisation) which increased the viscosity of cream also improved the whipping capacity. " The surface tension of milk and cream cannot be definitely related to whipping properties under all conditions, but the general relationship of decreased surface tension with improved whipping properties is true in the normal product."

The authors found that as the whipped cream began to stiffen the fat globules became distorted and grouped into clusters, which

[1] Cf. Müller, *Mitt. Lebensm. Hyg.*, **12,** 100 (1921).
[2] *J. Agr. Sci.*, **16,** 459 (1926).
[3] *Molkerei-Ztg.* (*Hildesheim*), **39,** 259 (1925).
[4] New York (Geneva) Agr. Expt. Sta., *Tech. Bull.*, **113** (1925).

appeared to be fairly uniformly distributed throughout the mass (Fig. 26).

They also observed that the whipping property of cream is increased as the percentage of fat present is increased. The probability that the clumping of the fat globules is a prominent factor in the structure and, therefore, the viscosity of whipped cream was experimentally demonstrated.[1] Pasteurisation of cream is known to reduce whipping capacity, and this fact is connected with the effect

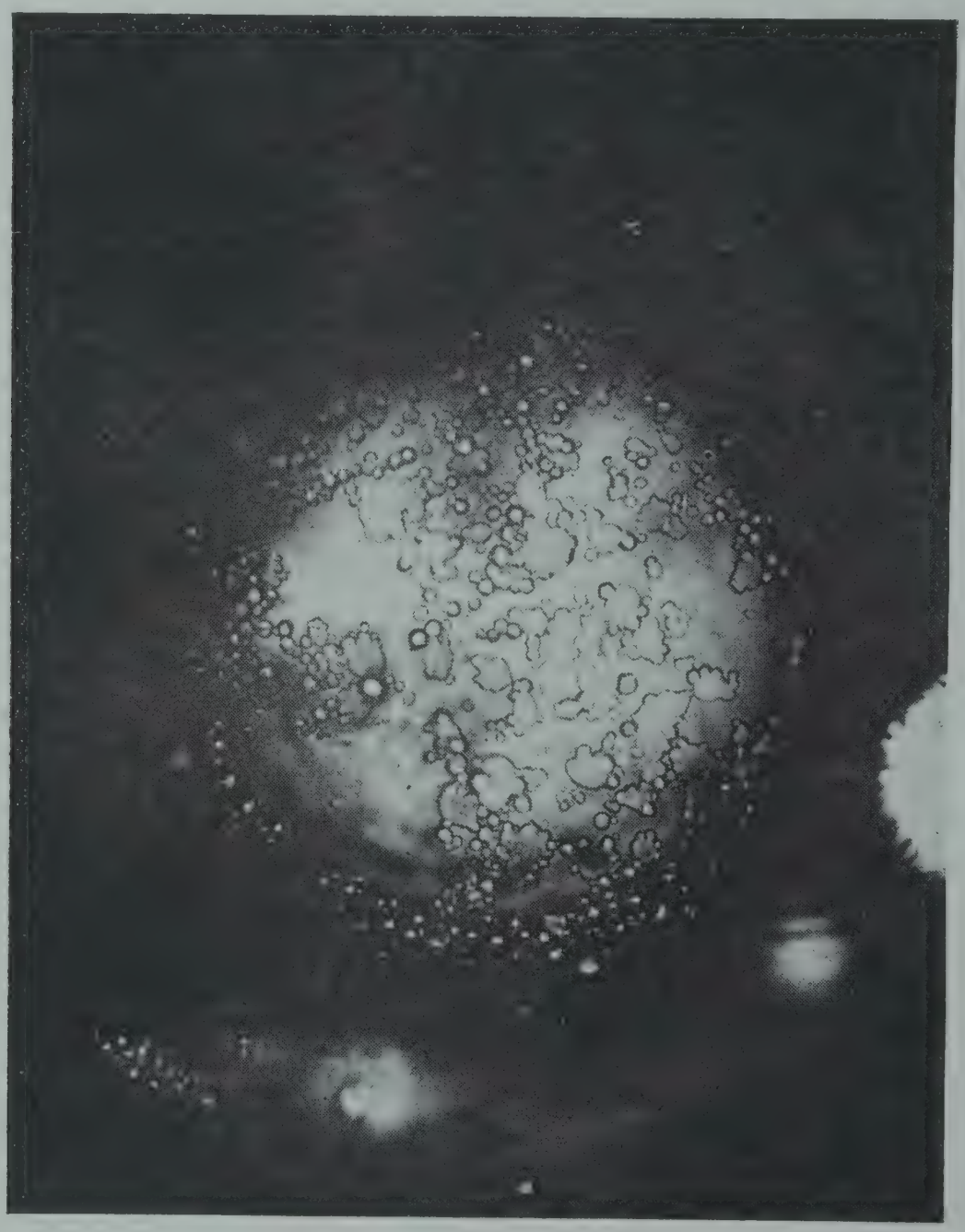


FIG. 26.—Photo-micrograph of whipped cream, showing a large air cell and the irregular fat clumps which interfere with the fluidity of the cream.

of pasteurisation in breaking up clusters of fat globules. Ageing of cream assists clustering and whipping (Fig. 27). The source of the cream is important, as the larger the fat globules therein, the more readily does clumping occur.

Homogenisation of cream reduces whipping property, this reduction being the more pronounced the higher the valve pressure. The logical explanation [2] is that due to homogenisation, the newly-

[1] *Milk Plant Monthly*, **15**, (3), 37 (1926). See also Rahn and Sharp, " Physik der Milchwirtschaft " (Berlin, 1928), p. 141.

[2] Clayton, *Z. deut. Öl-Fett. Ind.*, **46**, 321 (1926).

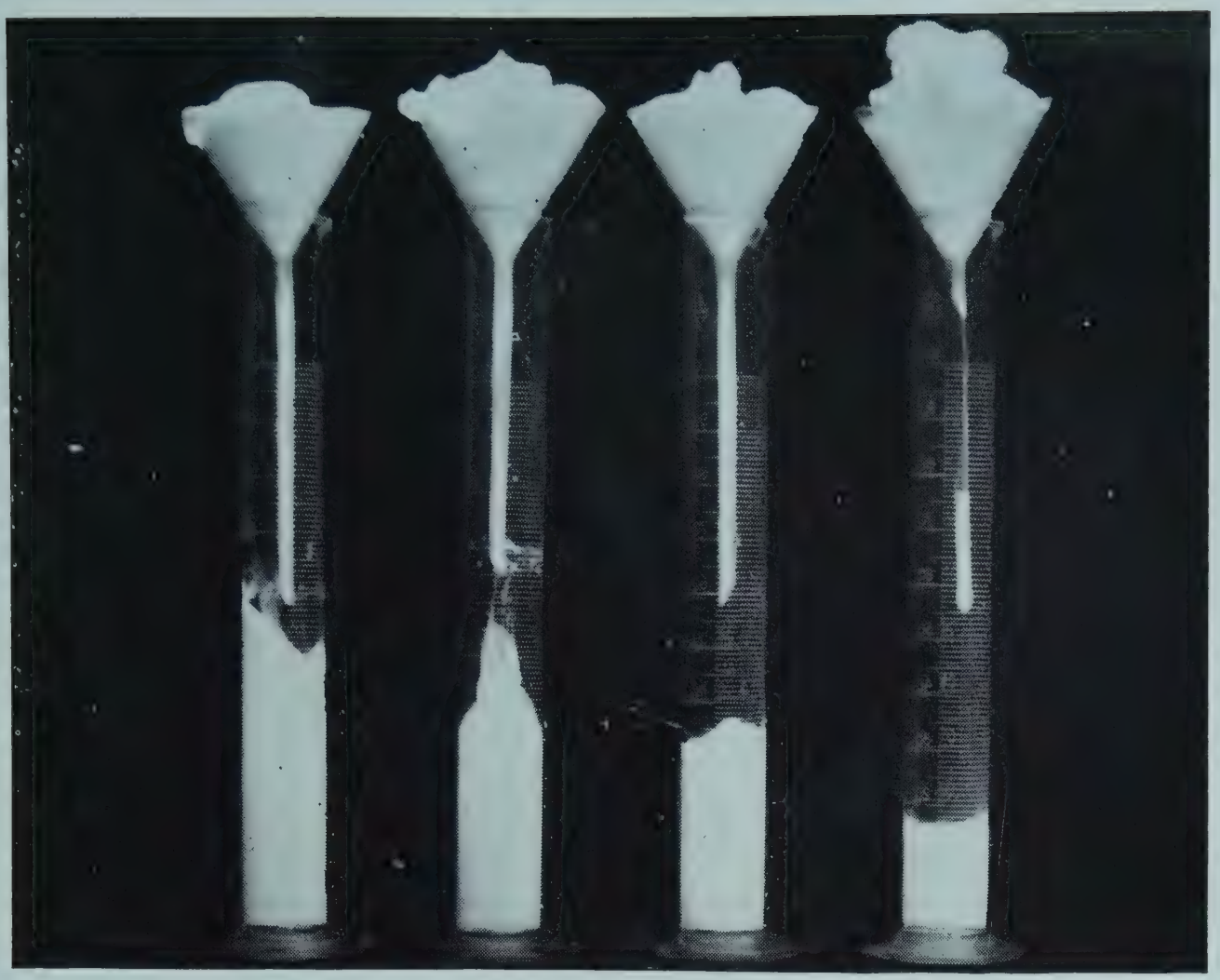


FIG. 27.—Whipped raw cream, with different percentages of fat.

Above : Holstein cream whipped when fresh.
Below : Holstein cream whipped after ageing.

[*To face p.* 232.

created enormous liquid/liquid interface adsorbs the proteins to so marked an extent that insufficient protein remains in solution to be available for adsorption at the air-liquid interface created by whipping. Addition of gum tragacanth *after* homogenisation restores the whipping capacity.

Homogenised Milk. The art of subdividing the fat globules in an emulsion to a smaller, more uniform size, is termed homogenisation.[1] The homogenisation of milk or cream is an important practice in dairy work, and the phenomena connected therewith beautifully illustrate certain familiar concepts in colloid physics and chemistry, particularly adsorption.

The subdivision of emulsified globules demands a force which increases enormously as the size of the globules decreases.[2] In 1892, Paul Marix [3] obtained two French patents in connection with the manufacture of margarine for making emulsions by passing the ingredients under pressure through a fine orifice. Later [4] he proposed that the liquids to be emulsified should be finely divided and intimately mixed by rubbing against a moving surface; under centrifugal force the liquids passed between a stationary plate and a rapidly-revolving plate. Julien [5] adopted the principle of homogenisation, but suggested forcing the liquids through a series of small holes. The subdivision of the globules in an already existing emulsion, such as milk, with a view to greater stability, was then conceived by Gaulin.[6] He employed a bundle of capillary tubes, against one end of which a powerful spring pressed a concave valve ball. A pressure of 250 atmospheres was required to force the milk (at 85° C.) through these tubes, whereby homogenisation was successfully achieved.[7] Later, agate was substituted for metal, owing to the excessive wear of the latter.[8]

Schröder and Wrede proposed a similar machine, but substituted for the tubes an agate shaft with spiral grooves cut in the surface, which caused the shaft to revolve as the milk was forced through under a pressure of 150 atmospheres. Schröder later replaced the revolving valve with an adjustable stepped valve, in which the face of each succeeding step was closer to the seat, thus providing for a gradual breakdown of the globules.[9]

In the commonly used Gaulin homogeniser is a triplex pump,

[1] Clayton, "The Theory of Emulsions and their Technical Treatment" (London, 1928), p. 175 *seq.*
[2] Hatschek, *Kolloid-Z.*, **7**, 81 (1910).
[3] French Pats., 218,946—7 (1892).
[4] French Pat., 221,583 (1892).
[5] French Pats., 220,446 (1892); 224,553 (1892).
[6] French Pat., 295,596 (1899); Brit. Pat., 22,875 (1904); U.S. Pats., 756,953 (1903); 753,792 (1904).
[7] Cf. also Bonnet, French Pat., 333,501 (1903) and Repin, French Pat., 328,064 (1902).
[8] Cf. Talansier, Brit. Pat., 19,626 (1909).
[9] German Pat., 277,225 (1912).

each cylinder possessing a suction and discharge valve, capable of withstanding very great pressure. In the discharge pipe is placed a rotatable agate valve, carefully ground into the seat and held close by a spring. The valve, which is comparable to a relief valve, is set to open at 2,000—3,000 lbs. per square inch. When, during working, the pressure reaches a point where the homogenising valve is forced open, the milk is spread over the face of the agate valve. The space between the valve and its seat is so narrow that the fat globules in the milk are burst and subdivided to a degree depending on the pressure employed, this being regulated by a hand wheel as required. As a rule, warm milk is homogenised, viz., at 60°—70° C. The subdivision of the fat globules is not to be attributed to any pulverising action of the valve but rather to (1) a shearing action consequent on the great velocity with which the milk passes through the valve, and (2) the atomising action due to the milk impinging on the wall of the valve. Doan points out that the second factor depends on the angle of impact, the greater this is the more pronounced the atomising effect. Maximum effect would ensue if the ground surface of the valve be as nearly as possible at right angles to the adjacent wall.

A comparison of the size-frequencies of the globules in homogenised milk and *skim milk* [1] has been given by Rahn,[2] and his data well illustrate the remarkable decrease in diameter of the fat globules due to homogenisation.

TABLE 46. SIZE-FREQUENCY ANALYSIS OF HOMOGENISED AND SKIM MILK.

Diameter-Range of Fat Globules.	Percentage of the Total Fat.	
	Homogenised Whole Milk.	Skim Milk.
μ		
0—1	1·2	1·7
1—2	83·6	40·6
2—3	15·2	30·8
3—4	*Nil*	13·4
4—5	*Nil*	8·0
5—6	*Nil*	6·0

The temperature at which homogenisation is conducted influences the size-frequency analysis, rise in temperature shifting the peak of the percentage of globules towards the small globules. The following table is due to Rahn.[3]

[1] Only the smallest fat globules present in whole milk are retained in skim milk (see p. 227).

[2] " Physik der Milchwirtschaft " (Berlin, 1928), p. 50.

[3] *Milchwirtschaft. Forsch.*, **2**, 382 (1925).

TABLE 47. INFLUENCE OF TEMPERATURE ON HOMOGENISATION.

Diameter-Range of Fat Globules.	Homogenisation Conducted at		
	20° C.	40° C.	65° C.
μ	Percentage of Total Milk Fat.		
0—1	2·3	1·9	4·3
1—2	29·3	36·7	74·4
2—3	23·3	21·0	9·0
3—4	29·8	25·2	12·3
4—5	*Nil.*	15·2	*Nil.*
5—6	15·4	*Nil.*	*Nil.*

The stability of homogenised milk with respect to the separation of the butterfat has been studied by Sobbe.[1] Two samples of milk were homogenised, and allowed to stand in a measuring cylinder at room temperature for seventy-two hours, a little formaldehyde being added as preservative. Analyses of the fat contents at different levels were then made, and the following results obtained :—

Sample.	Milk A.		Milk B.	
Initial Fat Content.	2·6 per cent.		3·3 per cent.	
Per cent.	Raw.	Homogenised.	Raw.	Homogenised.
Fat in lower, 50 c.c. . .	0·3	2·3	0·2	2·95
Fat in middle, 50 c.c. .	1·4	2·5	0·6	3·20
Fat in upper, 50 c.c. . .	8·5	2·9	14·5	3·85

Very little creaming takes place in the case of homogenised milk, whilst raw milk creams rapidly. Even centrifugal separation has but little effect on homogenised milk. Thus, Burr and Weise [2] found that whilst homogenised milk retained from 72·7 to 87·5 per cent. of the total fat after centrifuging, raw milk under similar treatment only retained from 3·8 to 7·3 per cent. of its total fat. The deformation of the fat globules has been studied by Baldwin.[3]

Cream is frequently homogenised, but skim-milk cannot be homogenised. Homogenised milk cannot be churned into butter, and homogenised cream cannot be " whipped " ; the addition of a colloid, such as gum tragacanth, is necessary if a permanent foam is desired. The Brownian movement of the fat globules is very marked, whilst there is a measurable decrease in the surface tension.

[1] *Milchw. Zentr.*, **43,** 503 (1914).
[2] *Molkerei-Ztg. (Hildesheim)*, **28,** 367, 381 (1914).
[3] *Am. J. Pub. Health*, **6,** 862 (1916).

The cause of these remarkable changes is the increased adsorption of milk proteins (notably casein) on the fat globules. Since in the homogenised products, the number of fat globules has increased about a thousand times, the adsorbing interface created is enormous. The adsorption of casein is at once manifested by the large increase in viscosity, homogenised milk and cream being much thicker than the original liquids. Wiegner [1] reduced the average diameter of the fat globules in milk from 2·9 μ to about 0·27 μ, and from viscosity measurements calculated that, of the casein in milk, 2·27 per cent. was adsorbed in the ordinary sample and 25·2 per cent. in the homogenised milk. (This is based on the assumption that only casein is adsorbed and that the mean thickness of the adsorbed layer is 6·8 $\mu\mu$). That adsorption is the main factor in homogenised emulsions has been demonstrated also by Briggs.[2]

Wiegner [3] has investigated the change in the diameter of the fat globules and the increase in their numbers when milk is homogenised. The following table shows the results obtained :—

Sample.	Density.	Per cent. Fat.	Diameter of Fat Globules.		Number of Fat Globules per 100 c.c.	
			Normal.	Homogenised.	Normal.	Homogenised.
			μ	μ		
1	1·0313	3·17	2·86	0·27	$2{\cdot}87 \times 10^{11}$	$3{\cdot}41 \times 10^{14}$
2	1·0320	2·87	2·94	0·17	$2{\cdot}40 \times 10^{11}$	$3{\cdot}02 \times 10^{14}$

The number of particles in the first sample has increased 1,188 times, and in the second sample 1,258 times, after homogenisation. The surface area of the fat globules has been increased 112 and 117 times respectively. The viscosities of the samples of raw and homogenised milks were in the ratio of 1 : 1·12 and 1 : 1·15 respectively. There was no apparent change in density and no difference in electrical conductivity, although Buglia,[4] in similar work, noted a slight increase in electrical conductivity consequent on homogenisation. Both Wiegner and Buglia report a slight decrease in osmotic pressure after homogenisation.

In the United States of America the term "viscolising" is usually substituted for "homogenising." There, a practice has been common of separating some or all of the cream from milk, viscolising such cream, and then remixing it with the residual milk or skim-milk. The resulting milk then gives rise to a greater volume of cream on standing, the milk in consequence seeming very rich in fat.

[1] *Kolloid-Z.*, **15,** 105 (1914).
[2] *J. Phys. Chem.*, **19,** 229 (1915) ; cf. Martici, *Arch. d. Fisiol.*, **4,** 133 (1907).
[3] *Kolloid-Z.*, **15,** 105 (1914) ; cf. Rousseau, *Le Nourrison*, **2,** 237 (1923).
[4] *Ibid.*, **2,** 353 (1908) ; cf. Touschot, *Rept. Min. Agr. Prov., Quebec* (1908), 184.

Now ordinary whole milk on homogenisation does not yield a bigger cream layer ; it is essential to viscolise cream and mix this with the milk. Thus, Martin and Combs [1] viscolised a sample of milk at 2,000 lbs. pressure and found that the volume of cream rising was 2 per cent., whilst the raw milk gave a cream volume of 10 per cent. On separating milk containing 4 per cent. fat and viscolising the cream and then reconstituting the milk, the following data was obtained relating to cream rising :—

TABLE 48. VOLUME OF CREAM RISING ON PASTEURISED MILK MADE FROM VISCOLISED CREAM AND UNTREATED SKIM MILK.

Treatment.	Temperature °C.	Per cent. of Total Volume appearing as Cream Hourly after Standing.											
		Hours.											
		1	2	3	4	5	6	8	10	12	14	16	18
Raw milk before separation, 4 per cent. fat . .	50	10	11	11	11	11	11	12	12	12	13	13	14
	35	10	13	18	21	21	21	21	21	21	21	20	20
Pasteurised milk made from 45 per cent. cream viscolised 2,500 lbs., 4 per cent. fat .	50	10	25	26	26	26	25	25	24	24	23	22	22
	35	0	0	31	31	31	31	30	29	29	29	28	27

Whilst offering no explanation for these results, the authors conclude " the fundamental cause in creaming ability of milk will possibly be found in the milk serum, and not the butterfat, as has long been the opinion."

Doan [2] has made an extensive study of viscolised reconstituted milk, being concerned with discovering a method for its detection. An examination of viscolised milk under the microscope showed the fat to be present as minute globules, " but gathered together into clumps containing hundreds of small globules. These clumps were extremely numerous, quite large in size and of irregular shape resembling flecks of foam. No normal globules were observable." Photomicrographs support Doan's statements.[3]

It was further observed that although the volume of the cream on viscolised reconstituted milk is so great, the concentration of fat in the cream layers is much less than in the cream on ordinary milk. In each case, the skim milk below had approximately the same fat content. Pasteurisation of viscolised reconstituted milk brought

[1] *J. Dairy Sci.*, **7,** 202 (1924).
[2] *Ibid.*, **10,** 501 (1927).
[3] *Vide* also Mortensen, Iowa Agr. Expt. Sta., *Bull.*, **180** (1918); Sherwood and Smallfield, *J. Dairy Sci.*, **9,** 68 (1926) ; Reid and Moseley, Missouri Agr. Expt. Sta., *Res. Bull.*, **91** (1926) ; Dahle, *Ice Cream Trade J.*, **26,** 55 (1930).

about a decrease in size of the " visco-clumps " and there was a greater number of individual small globules. The cream volume, which rises after pasteurisation, is still much greater in the case of the viscolised sample, though not so pronounced as in the unpasteurised sample.

Doan points out that it is not the fat content of the cream undergoing viscolisation, but the actual amount of cream so treated which is the important factor. " This would suggest that homogenisation sets up a structure of some sort among the ' visco-clumps ' of fat in the cream and that the volume of this structure depends on the available space at the time of homogenisation, and further that this structure maintains, to a certain extent, its volume upon dilution of the cream with fluid milk or skim milk." There the author leaves the matter.

Undoubtedly what happens is that homogenisation of the cream, which is so rich in milk proteins already adsorbed on the fat globules, still further increases the adsorption of protein due to the now greater fat/aqueous interface. Some sort of foam-like structure is possible if the adsorbed protein is " precipitated " as mechanical surface aggregates (Ramsden).[1] This structure will rise practically as a whole, owing to its lower density, when mixed with milk. No data is available regarding the gas content of the viscolised cream, although this factor is also important in the phenomena described above.

More recently, Doan [2] has published a lengthy account of further work on the problem. Mixtures were made from cream and milk or skim milk to the desired composition and exact analytical checks carried out. The fat content of the mixtures ranged from 3 per cent. to 16 per cent., and homogenisation was conducted at 38° C. at a pressure of 3,500 lbs. Clumping of the fat globules was followed by the microscope, and the factors involved were studied as follows : (*a*) fat concentration, (*b*) homogenising pressure, (*c*) heat treating the plasma, (*d*) acidity.

The table on p. 239, due to Doan, illustrates the general phenomenon under investigation.

It was found that " when other things are constant, the degree of fat-clumping increases with the concentration of the fat in the mixtures," thus agreeing with Hening [3] who found marked clumping for those mixtures containing over 10 per cent. of fat. The study of the influence of the pressure employed during viscolising showed that clumping began when 2,000 lbs. was developed, and increasing the pressure to 3,000 or 4,000 lbs. gave pronounced clumps in the mixtures so treated.

[1] *Z. physik. Chem.*, **47,** 336 (1904) ; *Trans. Liv. Biol. Soc.*, **33,** 3 (1919) ; cf. Freundlich and Loebmann, *Z. physik. Chem.*, Abt. A, **139**, 368 (1928).

[2] *J. Dairy Sci.*, **12,** 211 (1929).

[3] *Ibid.*, **11,** 299 (1928).

TABLE 49. INFLUENCE OF FAT CONTENT ON HOMOGENISATION RESULTS.

Sample.	Fat (per cent.).	Microscope Appearance of the Fat.	Cream Layer 48 hrs. at 10° C.	Fat in Upper Layer of Cylinder (per cent.)	Cream Layer on Diluted Sample 48 hrs. at 10° C. (per cent.).
1	4·2	Well dispersed—no clumps	*Nil*	4·3	*Nil*
2	7·2	Clumping tendency .	,,	7·15	,,
3	9·5	Evident clumping . .	,,	9·7	16·0
4	11·0	Very evident clumping .	,,	11·0	20·5
5	13·5	Prominent clumping .	,,	13·6	39·0
6	16·1	Pronounced clumping .	,,	16·3	68·0

In all cases the " Diluted Sample " means that the original sample was diluted with skim milk to a standard fat content of 3 per cent.

Various investigators [1] have shown that not only does the heating of milk destroy the creaming capacity of milk, but that this effect is more pronounced if the milk plasma is heated. Doan followed the effect of heating the *milk plasma* on the degree of clumping of the subsequently homogenised mixtures.

" A portion of fresh raw skim milk was divided into two parts, one of which was heated to 82·2° C. for 10 minutes and cooled. Fresh raw cream containing 53·4 per cent. fat was then standardised to approximately 12 per cent. fat, using the raw skim milk and again using the heated skim milk. Two other mixtures of approximately 13 per cent. fat were compounded in the same way." Doan then homogenised these four mixtures as usual at 38° C. and observed a reduction in the fat clumping in the two mixtures containing the heated plasma.

The effect of changing the acidity of the mixture to be viscolised was surprisingly slight, and led the author to conclude that the fat clumping was but little connected with the electrical charge carried by the fat globules.

The thermal stability of homogenised cream in its relation to " feathering " or " precipitating " in coffee or tea has been studied by Webb and Holm.[2] An increase in the pressure of homogenising the cream reduced the time for coagulation to occur in heating to 120° C. The acidity of the cream is the main factor involved in *thermal* stability. The two effects are probably related to the condition of the protein content of the cream, homogenisation and acidity both tending to " precipitate " adsorbed protein. The problem, however, is admittedly still in need of further investigation.[3]

Recently, there has been a development in the construction of

[1] Palmer and Anderson, *ibid.*, **9,** 1 (1926) ; Troy and Sharp, *ibid.*, **11,** 189 (1928).
[2] *Ibid.*, **11,** 243 (1928) ; cf. Burgwald, *J. Agr. Research*, **26,** 11 (1923).
[3] Recent work has been published by Tracey and Ruehe, Illinois Agr. Expt. Sta., *Bull.*, **352,** 569 (1930) ; *Creamery Milk Plant Monthly*, **19** (9), 36 (1930).

homogenisers, a two-stage valve machine becoming common practice. Two homogenising valves are arranged in series, each being set as required. The first is usually at high pressure (up to 5,000 lbs. per square inch), and the second at a lower (1,000 lbs. per square inch).

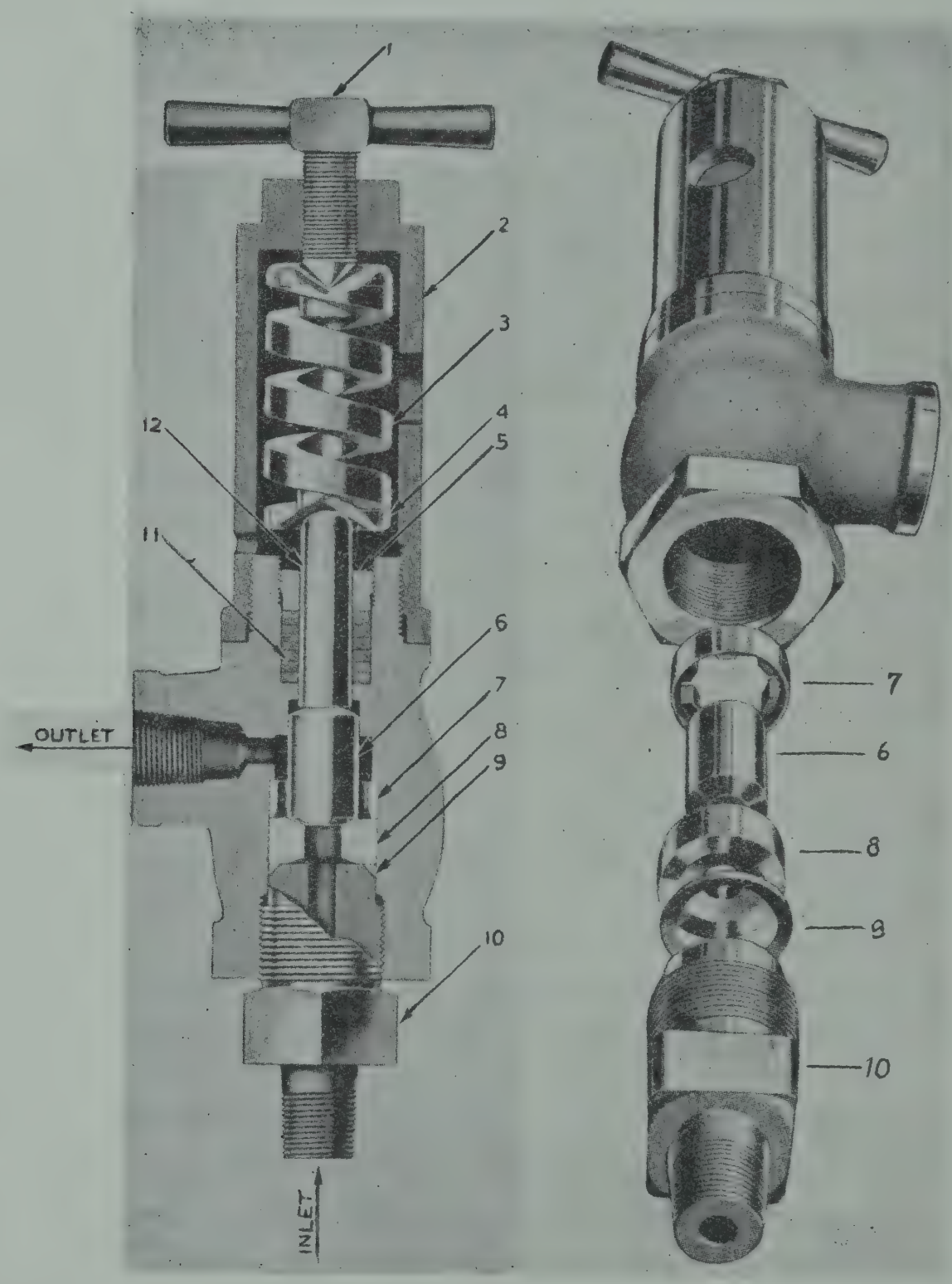


FIG. 28.—The Duo-Visco Valve.

1. Adjusting handle to adjust pressure.
2. Valve bonnet.
3. Pressure spring.
4. Spring end cup.
5. Packing gland.
6. Double-end viscolising valve.
7. Breaker ring and guide.
8. Double valve seat.
9. " Blow-proof " gasket for bottom plug.
10. Bottom plug.
11. Valve stem packing.
12. Valve stem.

The first valve permits intense subdivision of the fat globules and the second valve disperses the usual fat globule clusters common to homogenised emulsions.[1] By suitable regulation of the second valve, the viscosity of the homogenised emulsion can be altered as desired.[2]

[1] *Vide* Sherwood and Smallfield, *J. Dairy Sci.*, **9**, 71 (1926).
[2] Cf. Doan, *Food. Inds.*, **1**, 356 (1929).

Instead of two valves in series, the new Duo-Visco valve [1] may be employed. This is a single valve (Fig. 28), which may be set for any working pressure. The emulsion leaving the viscolising valve and containing fat globule clusters has to pass through a breaker ring, which redisperses the individual globules. In the present writer's experience the Duo-Visco valve has given excellent results.

COAGULATION OF MILK

Milk may be coagulated by heating, by adding acid and by the action of rennin. In each instance colloid-chemical changes occur as a result of the disturbance of the proteins—milk salts equilibrium.

Heat Coagulation. The results of heating milk, as in pasteurisation,[2] have importance scientifically and practically, *e.g.*, in relation to infant feeding,[3] as the subject is intimately connected with the question of the calcium content of milk.

In milk about 76 per cent. of the total calcium is combined with the casein ; the total calcium amounts to about 0·18 per cent. That portion of this element which is not united to the casein (about 24 per cent.) is present as phosphate and citrate.[4]

When milk is heated, the calcium content falls. Söldner [5] showed that a milk containing 18 mgm. of Ca per 100 c.c. when cold, lost17·5 per cent. of its Ca on boiling ; in two other instances the loss amounted to 18 per cent. and 24 per cent. respectively. Söldner inferred that the Ca was united with phosphorus as mono- and di-calcium phosphate, and on boiling was converted into the insoluble tri-phosphate.[6]

Grosser [7] filtered milk through a Bechold filter, removing the colloidal constituents. The filtered milk was analysed raw and after being boiled for 15 minutes. The CaO content of the raw filtered sample was 23·4 per cent. of the original (unfiltered) milk, and after 15 minutes boiling, only 18 per cent., or a loss of 5·4 per cent. CaO. Repeating the experiment with butter-milk he found no change in the CaO content of the raw and the boiled filtrates, and inferred that the phosphorus and calcium had been split off from the casein owing to the acidity. The experiments of Leighton and Mudge [8] on the heating of skim milk showed that the coagulation point coincides

[1] Made by the Cherry-Burrell Corporation, Chicago, Ill., U.S.A.
[2] Race, *Chem. and Ind.*, **42,** 166 (1923).
[3] Daniells and Stearn, *J. Biol. Chem.*, **61,** 225 (1924) ; Levine, *Arch. Pediatrics*, **44,** 273 (1927).
[4] Trunz, *Z. physiol. Chem.*, **40,** 263 (1903/04) ; Rona and Michaelis, *Biochem. Z.* **21,** 114 (1909).
[5] *Landw. Vers-Sta.*, **35,** 351 (1888) ; cf. Boekhout and de Vries, *ibid.*, **48,** 221 (1901).
[6] Cf. Palmer, *Proc. Soc. Exptl. Biol. Med.*, **19,** 137 (1921).
[7] *Biochem. Z.*, **48,** 427 (1913).
[8] *J. Biol. Chem.*, **56,** 53 (1923).

with an endothermic reaction, interpreted as absorption of heat due to the precipitation of Ca and Mg united with the milk proteins as citrates and phosphates.

According to Orr,[1] 26 per cent. of the total Ca of fresh milk is soluble, but in pasteurised and boiled milks (1 hour) this figure reduces to 20 per cent. and 15 per cent. respectively.[2] Similar figures were obtained by Magee and Harvey.[3] When milk is heated for 6 minutes at 106°—110° C. or for 30 minutes at 100° C., there is a decrease in soluble Ca, P and Mg, an effect which continues for 24 hours when the samples stand at ordinary temperature. No change in the pH value or the citric acid content, however, is observable.[4]

When milk is boiled, in addition to phosphate precipitation, the proteins undergo a change in their degree of dispersion, leading to clot formation.[5] According to Bardach,[6] complete coagulation of fresh milk at 100° C. requires 12 hours heating. Heating under pressure hastens coagulation, the time required at 130° C. being 60 minutes and at 150° C. only 3 minutes. Such data rule out lactalbumin as a thermal stabiliser for the casein system.

The total nitrogen in milk decreases steadily as the temperature of heating is raised. The lactalbumin remains practically constant until just over 70° C., but it decreases rapidly at over 80° C., and is practically entirely precipitated at 96° C.[7] Weinlig[8] found that after holding milk at 60° C. for 30 minutes, 8·5 per cent. of the lactalbumin became insoluble, whilst at 80° C., 40 per cent. became insoluble after 1 minute.

The question of the heat coagulation of milk has been investigated in considerable detail by Sommer and Hart,[9] who found coagulation to occur at 130°—150° C. according to the sample. For fresh milk there is no direct relation between the titratable acidity and heat coagulation ; pH is not the determining factor.[10] The main factor is the salt composition of the milk, particularly Ca, Mg, citrate and phosphate. Apparently, there is a marked optimum calcium content which confers optimum stability on casein, and the calcium content of the complex with casein is a function of the magnesium, citrate and phosphates present. Ca and Mg have effects antagonistic to that of

[1] *Scottish J. Agr.*, **9,** 377 (1926).
[2] Cf. Matsuo, *Osaka J. Med.*, **28,** 555 (1929).
[3] *Biochem. J.*, **20,** 873 (1916).
[4] Miss Daum, *Abs. Theses, Univ., Chicago, Science Series*, **4,** 423 (1925/26).
[5] Cosmovici, *Compt. rend. soc. biol.*, **91**, 649 (1924).
[6] *Monatsch.*, **18,** 199 (1897).
[7] Matsuo, *Osaka J. Med.*, **28,** 555 (1929) ; cf. Yamamura, *J. Agr. Chem. Soc. Japan*, **6**, 462 (1930).
[8] *Forsch. Geb. Milchw. Molkereiwes.*, **2,** 127, 175 (1922).
[9] *J. Biol. Chem.*, **40,** 137 (1920) ; cf. also Milroy, *Biochem. J.*, **9,** 215 (1915), McInerney, *J. Dairy Sci.*, **3,** 220 (1920) ; Leighton and Deysher, *Proc. World's Dairy Congress*, **2**, 1276 (1923) ; Sommer, *Butter and Cheese J.*, **20,** (xi.), 44 (1929).
[10] Rogers *et al.*, *J. Dairy Sci.*, **4,** 309 (1921) ; cf. Tapernoux and Katrandjieff, *Compt. rend. soc. biol.*, **101,** 828 (1929).

citrate and phosphate. An excess of either of the two classes of salts accelerates coagulation by heat. The authors [1] confirmed this conclusion and were able to remedy troublesome coagulation of evaporated milk on a commercial scale by the regulated addition of sodium citrate or disodium phosphate.

Whilst agreeing that ionic equilibria are intimately connected with the heat coagulation of milk, as with other colloidal systems, it has been shown that variations in the heat treatment of a milk profoundly influence the thermal stability of the *evaporated* product (condensed milk).[2] Temperatures up to 70° C. applied for 10 minutes result in diminished stability, whilst higher temperatures lead to a pronounced increase in stability. Homogenisation of the

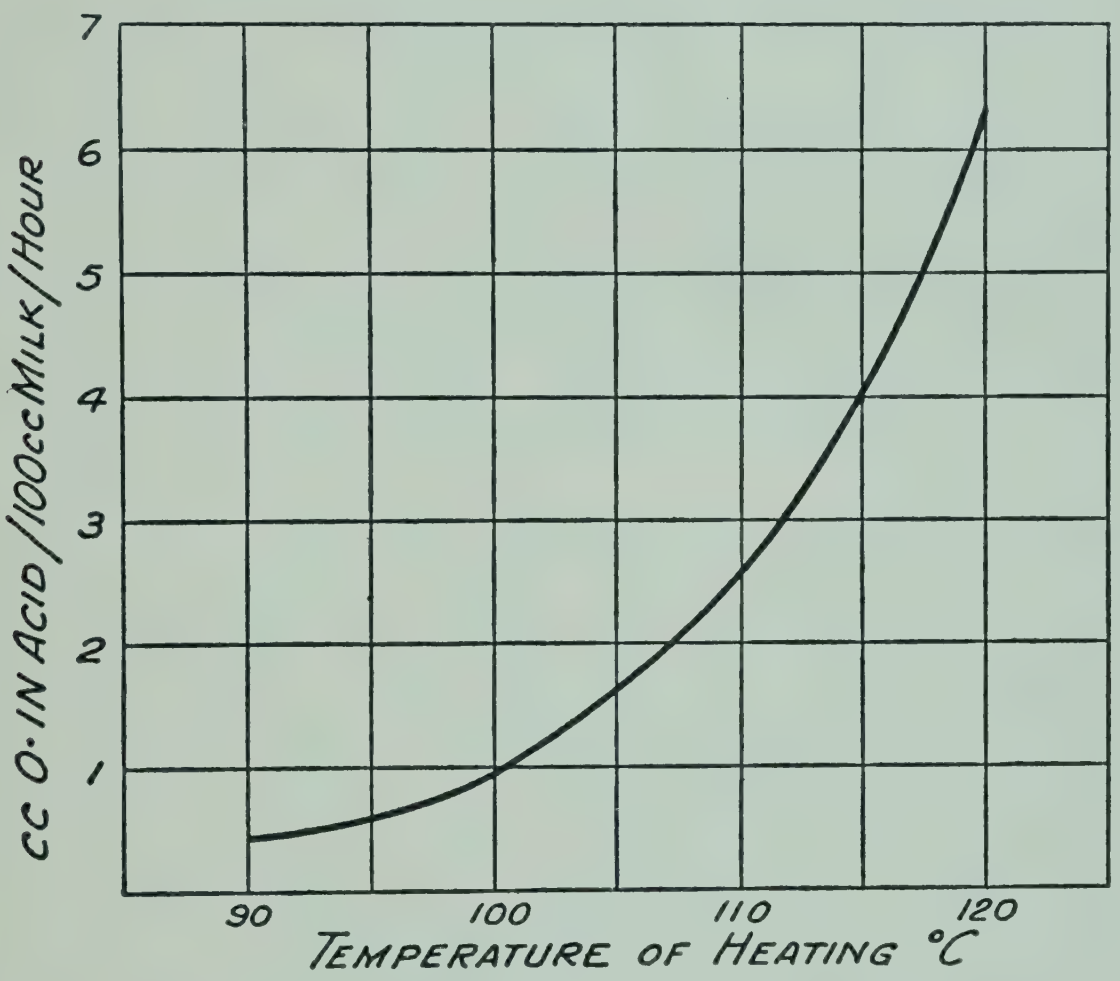


Fig. 29.—Relationship between the rate of acid formation in milk and the temperature of heating.

milk also assists thermal stability. It is obvious that considerable investigation must still be made into the nature of those variables concerned in arriving at an optimum balance between the pH and salt equilibria of milk before an adequate theory of heat coagulation can be presented.[3]

When milk is heated its acidity is increased,[4] but very little research has been reported on this other than by Whittier and Benton.[5] Working with skim milk at temperatures near boiling

[1] *J. Dairy Sci.*, **5**, 525 (1923).
[2] Deysher *et al.*, *ibid.*, **12**, 80 (1929). *Vide* Holm *et al.*, *ibid.*, **6**, 556 (1923).
[3] Benton and Albery, *J. Biol. Chem.*, **68**, 251 (1926).
[4] Milroy, *Biochem. J.*, **9**, 215 (1915); Cosmovici. *Compt. rend. soc. biol.*, **92**, 73 (1925).
[5] *J. Dairy Sci.*, **9**, 481 (1926).

point, they observed an initial decrease and then an increase in the titratable acidity,[1] although the pH value falls steadily. Loss of CO_2 accounts for the initial drop in titratable acidity, and the increase in acidity and fall in pH are due to the formation of acids from various constituents of the milk. Time of heating and temperature are factors in this connection. Buffer readjustments during coagulation decrease the rate of change in pH, and there is an uneven distribution of free acid between the whey and curd, as the latter adsorbs acid. Fig. 29 reproduced from a later paper by these authors [2] shows the relationship between rate of acid formation in milk and the temperature of heating.

Extending their investigations to milk heated above the boiling point, Whittier and Benton [3] showed that the rate of acid formation during heating is directly related to the time and temperature of heating and to the concentration of lactose, and is apparently unchanged by the coagulation of the casein. Lactose is believed to be the main source of the acid produced by heating milk, and in support of this contention it was shown that heating a 5 per cent. solution of lactose buffered with phosphate and citrate and adjusted to the initial pH, all as in fresh milk, gave rise to changes in pH and titratable acidity of the same order quantitatively as with heated milk.

Certain interesting data were obtained when heating milk in sealed cans at 110° C. and 120° C. The milk became flocculent at these temperatures after heating for 8 hours and 3·5 hours respectively, the pH then being about 5·54. The milk was curdled after heating for 8·5 hours and 4 hours respectively, the corresponding pH values being 5·52 and 5·45.

THE SOURING OF MILK

The souring of milk under the influence of micro-organisms has importance in the preparation of margarine.[4] When sufficient lactic acid develops the milk curdles and the " ripened " milk possesses a pleasing odour and flavour.

The physical chemistry of the process of souring has been investigated by Grimmer and Benduski,[5] Arnandi,[6] and van Slyke [7], qho has summarised the main findings.

Using separator skim milk at 18°—26° C. inoculated with a sour-milk starter it was found that in 72—96 hours the maximum decrease in lactose occurs, equivalent to about 28 per cent. of the lactose

[1] Van Slyke and Baker, *J. Biol. Chem.*, **40,** 335 (1919).
[2] *J. Dairy Sci.*, **10,** 343 (1927).
[3] *Ibid.*, **10,** 126 (1927).
[4] Clayton, " Margarine " (London, 1920), p. 46 *seq.*
[5] *Milchwirtschaft. Forsch.*, **7,** 76, (1928).
[6] *Boll. Ist Sieroterap. Milanese*, **7,** 387 (1928).
[7] N.Y. State Agr. Expt. Sta., *Tech. Bull.*, **140** (1928).

initially present. The casein coagulated in 24—30 hours, the titratable lactic acid then being 0·6—0·7 per cent. "When the milk is first visibly coagulated, about 13 or 14 per cent. of the casein is in the form of mono-calcium caseinate and 86 or 87 per cent. in the form of uncombined casein." As the acidity rises, the mono-calcium caseinate becomes free casein by loss of calcium.

Investigating the influence of souring upon insoluble calcium and inorganic phosphorus, it was found that as lactic acid formed, the di-calcium phosphate changes into soluble acid calcium or mono-calcium phosphate, whilst the calcium caseinate loses its calcium.

Sour milks possess no definitely characteristic pH value, the type of souring micro-organism influencing this, *e.g.*, *S. lactis*, pH 4·4—4, and *B. bulgaricus*, pH 3·86—3·7. The casein, however, commences to coagulate at about pH 4·64—4·78, over a time interval at 25° C. of 30—60 minutes.

COAGULATION OF MILK BY RENNET

When rennet is added to milk the calcium caseinate is acted upon and a separation into a jelly-like precipitate (curd) and liquid (whey) takes place. Rennet can clot 400,000 times its weight of casein in milk. Recent investigations by Fenger [1] show that rennet is capable of coagulating more than 2,000,000 times its weight of fresh milk in 10 minutes at 40° C. Shaking renders the rennet inactive,[2] a phenomenon investigated by Rideal and Wolf,[3] who find that the rennin is destroyed due to a chemical reaction occurring at the air-liquid interface, this being accompanied by a rise in surface tension and in the pH value of the solution. The chemical reaction takes place between the surface-active rennin and a surface-active reagent normally present in rennin solutions. This latter constituent is removable by dialysis or by selective adsorption on starch or Fullers' earth, and is in all probability a fatty acid. According to Korschun [4] an aqueous solution of rennin can be separated into fractions, having varying capacities for curdling milk, by filtration through porous clay.

Rennet acts best at 41° C. and the curd is then very firm ; a fluffy, soft curd results at temperatures between 15° C. and 20° C. (at which only 18 per cent. of the milk is curdled), and also at 50° C. (where 50 per cent. of the milk is curdled). The optimum range of temperature is from 36° C. to 45° C. The more dilute the rennet used the longer is the time taken in a firm clot being formed.[5]

[1] *J. Am. Chem. Soc.*, **45,** 249 (1923).
[2] Schmidt-Nielsen, *Z. physik. Chem.*, **69,** 547 (1909) ; *Z. physiol. Chem.*, **60,** 426 (1910) ; **68,** 317 (1910) ; Shaklee and Meltzer, *Am. J. Physiol.*, **25,** 81 (1909).
[3] *Proc. Roy. Soc.*, **A 106,** 97 (1924).
[4] *Z. physiol. Chem.*, **37,** 366 (1902).
[5] *Vide* Porcher, *Rev. gén. Coll.*, **7,** 260 (1929).

According to Grimmer and Kruger [1] the product of the concentration and the time of coagulation is not constant, but is an exponential function of the rennin (enzyme) concentration and follows a logarithmic curve. These results were confirmed by Grimmer and Rudzik,[2] who found the maximum of the logarithmic curve to flatten with decreasing pH value.

When milk is heated the action of the rennin is delayed, owing possibly to the precipitation of calcium, but if $CaCl_2$ be added, normal coagulation occurs. Milk deficient in calcium salts requires a longer time for rennet coagulation to take place.[3]

If $CaCl_2$ be added to milk immediately after the rennet, coagulation is favoured by small amounts and retarded by larger amounts. Should the $CaCl_2$ be added after rennet coagulation has set in, the time necessary for the clot is lengthened according to the time the addition is made and to the amount of $CaCl_2$ added, and such retardation is made evident even with those small amounts of $CaCl_2$ which, if added prior to the onset of coagulation, would hasten this. Hence, the later influence of the $CaCl_2$ is believed [4] to be due to an inhibitory reaction on the rennin and to a change in the precipitability of paracasein.

The work of Rüdiger and Worster [5] fixes 0·142 per cent. of added $CaCl_2$ as the upper limit, thereafter inhibition of coagulation is seen. Addition of $CaCl_2$ to milk results in a continuous but diminishing lowering of the pH value, but this acid effect is not the only factor concerned in the effect of $CaCl_2$ on coagulation. More likely, $CaCl_2$ causes a shift in the acid-base equilibrium by a change in the phosphate equilibrium.

In this connection, Whittier [6] has shown that the maximum buffering in normal milk occurs at about pH 5·50. From the difference in the buffer action of whole milk and acid whey, it follows that the buffer action of casein occurs in the pH range 4·5—5·7, being maximal at pH 5·2. The casein of milk and various prepared caseins possess different buffer capacities. Since the addition of rennet to milk brings about the disappearance of the pronounced maximum buffering at pH 5·5, Whittier believes casein is converted by rennin to a substance with several dissociation constants.[7]

Interesting observations were made by Rupp,[8] who reported that coagulation by rennet was more rapid between 55° C. and 65° C.

[1] *Milchwirtschaft. Forsch.*, **2,** 457 (1925).
[2] *Ibid.*, **3,** 361 (1926). The results were confirmed by Mundinger, *ibid.*, **4,** 369 (1927).
[3] Söldner, *Landw. Vers-Sta.* (1888), 351 ; Ringer, *J. Physiol.*, **11,** 464 (1890) ; Conradi, *Munch. med. Wochenschr.*, **48,** 175 (1901) ; Kreidl and Lenk, *Biochem. Z.* **36,** 357 (1911).
[4] Rona and Gabbe, *Biochem. Z.*, **134,** 39 (1922).
[5] *Ibid.*, **216,** 367 (1929).
[6] *J. Biol. Chem.*, **83,** 79 (1929).
[7] Cf. Pertzoff, *J. Gen. Physiol.*, **10,** 1002 (1927).
[8] U.S. Dept. Agr., *Bull.*, **166** (1913).

than with raw milk, but slowed down at about 70° C. to nearly double the time ; in this case, however, a finer grained coagulum occurred. Dominicis and Rotonda [1] found that heating milk to over 75° C. decreased the action of rennet and finally inhibited it. This they considered due to a progressive agglomeration of the casein particles, which thus pass into the state preceding separation by coagulation, *e.g.*, as by acidifying. Rennet preserves the homogeneous dispersion of casein, promoting smooth gelatinisation, hence the antagonistic effect of heating.

Although boiled milk is not curdled by rennet, coagulation occurs on the addition of small amounts of $CaCl_2$. Lindet [2] suggests that this is due to the interaction of the $CaCl_2$ with the alkali phosphate and citrate in the milk, thus reducing the content of these, and rendering the casein less soluble. Bergen [3] investigated the influence of the addition of NaCl and $CaCl_2$ and reported that the results were irregular and that no theoretical explanation was available. The need for calcium in the rennet coagulation of milk is now generally accepted as the result of numerous investigations.[4]

According to Mattick and Hallett,[5] when milk is heated to 41·2°—43·4° C., rennet coagulation occurs in 3 minutes even if the rennet is not added for 5 hours. On heating the milk to 90·6°—92·3° C. the rennet requires to be added within 30 minutes, whilst heating to 96·2°—98·4° C. destroys the capacity of the milk to curdle with rennet. No change in the diffusibility of N was observed consequent on these heatings, but the diffusibility of P was reduced after heating for 30 minutes at 79·5° C., whilst heating even at 51·7° C. soon reduced the diffusible Ca.

Just what is the relation between temperature and rennet clotting in relation to the constituents of the milk is still a field for research. Undoubtedly the thermal stability of colloidal calcium caseinate is involved,[6] as are factors connected with paracasein, *e.g.*, the influence of cations on the electric charge. Systematic experiments at 35° C. under carefully controlled conditions, led Dominicis and Rotonda [7] to conclude that rennet coagulation of milk does not depend on substances present in the whey but only on the physical condition of the casein. On this basis, phosphates and calcium compounds do not play any part in the mechanism of coagulation,

[1] *Ann. v. scuola agr. Portici*, **17**, (ii.), 1 (1922).
[2] *Compt. rend.*, **157**, 381 (1913).
[3] *Chem. Weekblad.*, **20**, 479 (1923).
[4] Arthus and Pages, *Arch. Physiol.*, **2**, 331, 540 (1890) ; Bang, *Skand. Arch. Physiol.*, **25**, 105 (1911) ; Cosmovici, *Bull. soc. chim. biol.*, **7**, 124 (1925) ; Milroy, *Biochem. J.*, **9**, 215 (1915) ; Ringer, *J. Physiol.*, **11**, 464 (1890) ; Van Dam, *Z. physiol. Chem.*, **58**, 295 (1908) ; Van Slyke and Bosworth, *J. Biol. Chem.*, **14**, 203 (1914) ; Palmer, *Proc. Soc. Exptl. Biol. Med.*, **19**, 137 (1921) ; Koestler, *J. Dairy Sci.*, **8**, 28 (1925).
[5] *J. Agr. Research*, **19**, 452 (1929).
[6] Michaelis and Marui, *Aichi J. Exptl. Med.* (*Tokyo*), **1**, 45 (1923).
[7] *Ann. chim. applicata*, **16**, 294 (1926).

and the real reason why boiled milk does not clot with rennet is because of a change in the colloidal state of the casein, this losing its tendency to swell and agglomerate.

During the action of rennet there is no change in the hydrogen ion concentration, but this is raised by addition of soluble calcium salts, and decreased by addition of ammonium oxalate. It is of interest in this connection to note that rennet acts best in acid solution, the optimum action being at pH = 6·0.[1] However, Porcher,[2] who has been a prominent investigator in this field, has given the following results, showing the influence of the acidity of the milk on the velocity of clotting with rennet :—

Time (mins.).	pH.
17′	6·43
9′ 15″	6·39
5′ 40″	6·32
4′ 45″	6·12
3′ 55″	6·01
2′ 40″	5·90
1′ 55″	5·83

Milks vary in their capacity for such curdling and samples with the same titratable acidity will show different times for rennet clotting. Palmer [3] believes " it may be best to consider that the zone of maximum coagulability is the isoelectric zone of calcium paracaseinate in the presence of calcium ions," a view supported by investigations of Michaelis,[4] showing that cations (particularly bivalent) displace towards neutrality the isoelectric points of electronegative ampholytic colloids.

Van Dam [5] found that the coagulation time varied inversely as the hydrogen ion concentration. There is evidence that the clotted milk has about 5 per cent. increase in surface tension as compared with raw milk.[6]

THEORIES OF RENNIN COAGULATION

Mellanby [7] advanced the interesting theory that the clotting of milk by rennet was due to the adsorption of the enzyme by the casein and the subsequent precipitation of the enzyme-casein complex by the bivalent Ca ions present in milk. The quantity of ionised Ca salt required to effect precipitation is intimately related

[1] Michaelis and Rothstein, *Biochem. Z.*, **105,** 60 (1920).
[2] *Rev. gén. Coll.*, **7,** 267 (1929).
[3] " Fundamentals of Dairy Science " (New York, 1928), p. 208.
[4] *Biochem. Z.*, **94,** 225 (1919) ; *Kolloid-Z.*, **30,** 209 (1922).
[5] *Z. physiol. Chem.*, **58,** 295 (1908).
[6] Cosmovici, *Compt. rend. soc. biol.*, **90,** 1313 (1924).
[7] *J. Physiol.*, **45,** 345 (1912).

to the quantity of the enzyme adsorbed. The view of Wright [1] is that coagulation is due to the colloidal dispersion of the caseinogen being altered in such a manner that precipitation by bivalent metal ions is facilitated. It is generally agreed that caseinogen and casein produced therefrom by rennet action are not only different physically but chemically.[2]

Bang [3] observed that by adding rennet in fractions to milk, summation is noted, *e.g.*, the coagulation time on adding 0·1 c.c. of rennet to 10 c.c. milk is 8 minutes, and on adding 0·2 c.c., 4 minutes. If, however, 0·1 c.c. is added, and 4 minutes later another 0·1 c.c., then clotting occurs 2 minutes after the second addition. No such summation occurs if the milk, after the first addition of rennet, is heated to 65° C. He concluded that rennet is not a coagulating enzyme, since rennet of itself does not produce curdling, and the final act of clotting is not true coagulation but is more akin to the precipitation of protein by neutral salts.

Another conception of the rennet coagulation of milk is given by Schryver.[4] When solutions of calcium salts and sodium cholate are mixed, a clot results on warming. For salts which increase the surface tension of water, the greater their amount present, the shorter will be the time required for clotting to take place. Salts which lower the surface tension decrease the time for coagulation only up to a certain limit of concentration, above which the time is increased, or the coagulation entirely prevented.[5] The inhibition of coagulation is attributed to the adsorption of simple molecules by the more complex colloids, which are thereby prevented from coalescing. In milk the necessary materials exist, but the adsorption of simple molecules from the solution inhibits the coalescence. Schryver assumes that the enzyme clears the surface of the colloid from the adsorbed substances, thus permitting coalescence to occur. He asserts that in milk the clot formation depends on the presence of four series of substances present : simple inhibitory substances, colloids, enzymes and calcium salts. Recent work [6] shows that bile and bile salts are inhibitory substances.

Alexander [7] advanced a somewhat similar idea, his view being that the casein is protected or held in stable suspension by the lactalbumin, which rennet destroys, thus permitting coalescence and precipitation of the casein particles. Such a view is now untenable in view of the work done on the influence of protective

[1] *Biochem. J.*, **18,**, 245 (1924).
[2] Zaykovskii, *Fermentsforchung*, **8,** 537 (1926) ; Pertzoff, *J. Gen. Physiol.*, **10,** 987 (1927) ; **11,** 253 (1927).
[3] *Skand. Arch. Physiol.*, **25,** 105 (1911).
[4] *Proc. Roy. Soc.*, **B 86,** 460 (1913).
[5] Cf. Doyon, *Compt. rend, soc. biol.*, **83,** 918 (1920).
[6] *Vide* Clementi, *Arch. fisiol.*, **23,** 475 (1924).
[7] *Eighth Intern. Cong. Appld. Chem.*, **6,** 12 (1912) ; *Ind. Eng. Chem.*, **15,** 254 (1923) ; **16,** 974, 1282 (1924).

colloids like gelatin and gum arabic on rennin action. Their presence favours clotting and tends to a firmer gel.[1] Fuld [2] showed that milk diluted with milk serum (neutralised filtrate after acid coagulation) set with rennin in the same time as did milk diluted with milk serum pre-digested with rennin.

A splendid summary of the literature has been made by Palmer and Richardson,[1] who also carried out extensive experimental work. They sum up the position very succinctly as follows : " It is not possible to understand correctly the clotting of milk by rennin by considering it from any one point of view. Both phases of the phenomenon, the action of the colloidal rennin on the caseinates, and the clotting of the resulting paracaseinate, are colloidal reactions. At least three important questions stand out in connection with rennin action, none of which can as yet be answered with complete satisfaction : What is the exact character of the colloidal calcium caseinate in milk ? What is the nature of the change in this colloid which causes its increased affinity for cations ? How does the colloidal enzyme rennin produce this change ?

" The ultramicroscopic evidence and the precipitation by electrolytes appear to indicate that calcium caseinates and paracaseinates are true suspensoids at the pH of milk. There is much less certainty regarding the nature of the change in the particles of calcium caseinate which increases their affinity for cations with a corresponding rise in sensitivity. The nature of this change remains to be determined, but it cannot be a simple molecular division.

" The question of the method by which minute amounts of colloidal enzyme can effect so profound a change in so short a time must at present remain on the unsatisfactory basis that it does so during a temporary adsorption on the surface of the calcium caseinate particles.

" The second phase of the rennin phenomenon rests on much firmer colloidal grounds. The ultramicroscopic evidence and the accumulation of experimental observations definitely point to the conclusion that the rennet clot is an unstable, rapidly synerising gel of amorphous particles of calcium paracaseinate, each retaining its identity. The laws governing the formation of this gel are identical with those recently summarised by Weiser for jellies formed by precipitation from a sol. The type of precipitation reaction is one involving the effect of valency of the precipitating ion . . . there is also a definite temperature range for the calcium paracaseinate gel. Temperature is also a factor in the rapidity of its syneresis. We have not yet determined the calcium combining capacity of casein and paracasein at the optimum clotting temperature, but it is

[1] Palmer and Richardson, *Am. Coll. Symp. Monog.*, **3,** 118 (1925) ; cf. also the recent extensive review by Porcher, " Le Lait au Point de Vue Colloïdal " (Lyon, 1930).

[2] *Beitr. Chem. Physiol. Path.*, **2,** 169 (1902).

reasonable to suppose that the affinity for calcium increases with rise of temperature. This would account for the greater instability in the presence of soluble calcium salts. The rule seems to be that the greater the calcium combining capacity of the particles is and the farther removed they are from saturation with calcium, the less calcium ions are required to neutralise their electric charge. This undoubtedly explains in part why sodium ions will clot the calcium paracaseinate formed in milk by rennin action."

Marui [1] has shown that the decreased rennin coagulability of casein due to boiling, does not depend upon an increased calcium-binding power of the casein. As regards the effect of calcium in the rennin action he finds that univalent cations cannot reproduce the calcium results, whereas all bivalent and tervalent cations can. Again, there is the fact that any suspension which is stabilised by casein as a protective colloid can be precipitated by rennin. The micallæ so protected may be of any insoluble material, including insoluble calcium salts. Rennin so affects the casein in the protective film as to reduce its protective action and the now unstable colloid is precipitated.

Apropos of this, Porcher [2] has shown that the complex Ca caseinate—Ca phosphate is an association of two colloidal systems, the former acting as a protective colloid to the latter. The fact that heating alters the complex so that it is no longer coagulable by rennet was experimentally determined as due to the greater heat-sensitiveness of the phosphatic micelles. Later Porcher [3] heated equal portions of Ca caseinate at pH 9·76 to 40°, 65° 80° and 100° C. for 30 minutes. The solutions were cooled to room temperature and H_3PO_4 added, thus forming some Ca phosphate. Rennet was added and the following results obtained :—

Heated at	Final pH.	Curd Result.
40° C. and 65° C.	7·13	Firm in 310 seconds.
80° C.	6·97	Less firm in 470 seconds.
100° C.	6·94	Soft after 720 seconds.

Here the alteration of the Ca caseinate micelles by heating has restrained curd formation.

CHEESE

Cheese is a milk food, casein being the important chemical substance involved. Some typical analyses illustrating the system to be studied are reproduced from Plimmer.[4]

[1] *Biochem. Z.*, **173,** 363 (1926).
[2] *Compt. rend.*, **182,** 1247 (1926).
[3] *Ibid.*, **182,** 1420 (1926).
[4] "Analyses and Energy Values of Foods" (London, 1921), p. 85. See also Doane and Lawson, U.S. Dept. Agr., *Dept. Bull.*, **608** (1928).

TABLE 50. ANALYSES OF CHEESE.

Variety.	Water.	Protein.	Fat.	Salt.	Total Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Little Wilts (soft) .	41·1	22·1	30·3	1·4	3·4
English Cheddar .	33·7	25·2	33·4	1·8	4·4
Cheshire . .	34·9	22·7	33·8	2·1	4·9
Caerphilly . .	43·0	27·2	13·4	2·1	5·2
Stilton . .	24·6	26·3	42·8	2·4	3·8
Parmesan . .	26·4	33·7	34·9	2·6	6·6

The various stages [1] in the manufacture of Cheddar cheese, apart from bacteriological operations, are : (1) coagulation of the milk, (2) cutting the clotted milk, (3) agitating and heating the curd, (4) draining the curd, (5) uniting ("cheddaring") the cut curd, (6) milling the curd, (7) salting and pressing the curd, (8) ripening the cheese

The milk is clotted [2] chiefly by the use of rennet and the clot is later cut into pieces so as to permit the escape of whey. The cutting is correctly timed so that the curd is neither too soft (when fat would be lost) nor too hard (when whey would be retained) and the curd is cut into : (*a*) cubes of about $\frac{1}{4}$ to $\frac{1}{2}$ inch side (for Cheddar cheese), (*b*) small grains (Swiss cheese), (*c*) coarse pieces (Camembert cheese). By cutting the curd, whey is more readily expelled and subsequent heating is facilitated.

Heating accelerates whey separation, rendering the action of rennet more effective and increasing the production of lactic acid, with a consequent toughening of the curd. The duration of heating depends on the acidity of the whey and varies from 60 minutes for 0·12 per cent. lactic acid to 20 minutes for 0·15 per cent. acid. The temperature to which the curd is raised depends on the variety of cheese made, the fat content of the milk, and the acidity of the whey. The lower the temperature employed the smoother is the texture of the cheese. The temperature may be taken as high as 38° C., *e.g.*, in making American Cheddar cheese.

After removing the whey, the pieces of curd are matted together, thus controlling the moisture (whey) content, and assisting in producing a plastic curd. The matted curd is subsequently milled, thereby permitting an even incorporation of salt, the expulsion of more whey and the escape of undesirable gases. The addition of

[1] For a detailed account see "Cheese," by Van Slyke and Price (New York, 1927), Part 3. An extensive bibliography is included, p. 330 *seq.*

[2] Regarding the different types of milk and their relation to clotting with rennet see Koestler, *J. Dairy Sci.*, **8**, 28 (1925).

salt aids in the expulsion of whey and hardens and contracts the curd. Finally, the cheese is pressed and stored for maturing.

The details of cheese manufacture [1] vary with the many kinds of cheese of both the hard and soft varieties, but the foregoing brief description shows that colloid chemical problems arise throughout the manufacture.

The structure of cheese varies from a dough-like to a granular texture, of varying degrees of hardness. The physical chemistry of the subject is mainly concerned with the transformation of the delicate milk clot into the more or less tough or elastic mass represented by cheese. Just what are the changes in structure undergone during the preparation of cheese is even yet a matter for investigation, whilst there is no published work on the size-frequency analysis and the functions of the fat globules present.

Cheese is a colloid system and its texture reflects the action of electrolytes (mainly acids) on the constituents. Casein, of course, is the fundamental constituent and its importance is due to :—

(i.) the entanglement of fat globules as the clotted milk solidifies,
(ii.) the retention of whey in the curd,
(iii.) the subsequent changes in the casein due to bacterial and enzyme action.

Casein can hold 1·25 times its weight of water and the curd in the final cheese confers firmness and solidity over a wide temperature range. According to Chick and Martin,[2] in the case of both alkaline and acid casein solutions, the viscosity increases with the amount of alkali or acid present, which indicates that the casein-salt complex has a greater absorptive power for water than has casein itself.

Acidity has a marked influence on texture, and the excessive amount of free lactic acid present in Edam cheese is the direct cause of its brittle character.[3] The moisture content, too, influences texture, too little moisture giving a dry, crumbly cheese, excess moisture a soft cheese.[4] Watson [5] studied the relation between the pH and the texture of Emmenthal or Swiss cheese, finding that the choice of the bacterial flavouring starter is important in controlling the pH and texture of the cheese. His work is preliminary, but he concludes that " variations in the colloidal structure of the cheese, as shown by the difference in the binding of the water, exert an important effect on the texture."

An important contribution to the subject was made by Allemann

[1] Problems relating to processed cheese are discussed by Templeton and Sommer, *J. Dairy Sci.*, **13,** 203 (1930) and Sommer, *ibid.*, **13,** 288 (1930).
[2] *Kolloid-Z.*, **11,** 102 (1912).
[3] Boekhout and de Vries, *Rev. gén. lait.*, **7,** 385 (1909) ; Van Dam, *ibid.*, **8,** 169 (1910) ; **9,** 151 (1911).
[4] Cf. Van Slyke, N.Y. (Geneva) Agr. Expt. Sta., *Bull.*, **207** (1901).
[5] *J. Dairy Sci.*, **12,** 289 (1929).

and Schmid,[1] who investigated the elasticity of the coagulum produced in milk by rennet. An apparatus was employed which measured the resistance which the curd offered to a vertical rod carrying three concentric rings. Thus they were able to investigate the effect of time, concentration of rennet, concentration of acid, and other factors, on the curd produced. It was found that the elasticity of the curd increased in direct proportion to (*a*) acidity of the milk, (*b*) concentration of the rennet, (*c*) addition of soluble calcium salts, (*d*) rise in temperature of setting. No apparent maximum of elasticity was observed with increasing temperature, though the time necessary for coagulation reached a maximum at 41° C. Further work by Allemann,[2] however, showed that with increasing temperature, the consistency of the curd increases to a maximum at 41°—42° C. and thereafter the curd becomes rapidly softer. The following table by Allemann illustrates this effect, the "basic consistency" being the value found with his apparatus at 20° C.

TABLE 51. CONSISTENCY OF RENNET CURD OF MILK.

Experiment.		Basic Consistency.	Temperature (°C.) of Experiment.								
			20	25	30	35	40	42	45	47	50
2	Clotting time (mins.)	—	25·2	16·7	12·2	9·7	8·5	8·3	8·6	9·4	13·5
	Consistency . .	11	11·0	22·0	33·0	44·0	55·0	55·0	44·0	?	0
3	Clotting time (mins.)	—	27·8	19·3	14·0	11·8	10·4	10·6	11·1	13·0	22·1
	Consistency . .	6·5	6·5	13·0	19·5	26·0	32·5	32·5	26·0	—	0

Agitation during clotting inhibited the velocity about 8 per cent. With cold milk, a longer time was required for clotting, and the resulting curd had a decreased elasticity.[3] The work of Allemann and Schmid requires extension to include the changes occurring when the curd is treated as for cheese.

Marquardt [4] has carried out an extensive investigation of the physical chemistry of soft cheese manufacture. The curds were prepared by a Neufchatel process or a slight modification of this. For example, a cream of 15 to 18 per cent. fat content was pasteurised at 82·2° C. for 30 minutes and homogenised at 43·3° C. under 2,500 lbs. pressure. The cream was then set with a bacterial starter and rennet, and after 12 to 18 hours the curd was warmed to 34·4°—43·2° C. (depending on the nature of the curd) for 2—4 hours. The drained curd was then salted, using 1·25 per cent. of salt.

[1] *Landw. Jahrb. Schweiz.*, **30,** 357 (1916).
[2] *Kolloid-Z.*, **24,** 27 (1919).
[3] Cf. Guittonneau, *Compt. rend.*, **180,** 1536 (1925).
[4] *J. Dairy Sci.*, **10,** 309 (1927).

In the author's wide range of experiments, milk and cream of widely varying fat contents were used. The following important factors were studied : (1) homogenisation pressure, (2) rennet concentration, (3) temperature of setting, (4) composition of the milk or cream, (5) heat treatment of the curd.

Homogenisation of pasteurised milk (average 5 per cent. milk fat) at 54·4° C. resulted in a smooth texture and reduced the grain-size of the fat globules. A pressure of at least 2,000 lbs. was necessary. Homogenisation of skim milk, as expected, did not improve the texture of the curd. The relation between the homogenised fat globules and the texture of the cheese is given by Marquardt in the following table :—

TABLE 52. INFLUENCE OF HOMOGENISATION ON CHEESE TEXTURE.

Factors Studied.	Homogenisation Pressure.		
	None.	2,500 lbs.	4,500 lbs.
Average size of fat globules .	3·88μ	1·56μ	0·97μ
Percentage of fat globules larger than average for normal milk used	54·20	3·40	0·00
Cheese texture . . .	Coarse	Smooth	Very smooth

According to this worker one cannot correlate the smooth texture of a soft cheese to the fine dispersion of the fat globules due to homogenisation. "Nor is it possible to associate the size and number of fat clumps resulting from homogenisation directly with the smooth texture of the resulting cheese. Cheese made from cream containing high percentages of fat is smoother in texture than a cheese made from a thinner cream, due to the additional fat. This is true regardless of the size of fat globule clumps and their number."

Regarding the amount of rennet used, variations from 1·5—60 c.c. rennet per 1,000 lbs. of milk, showed no material effect in the initial drainage of whey from the curd. Excess rennet tends to a drained curd of rather dry appearance, and sometimes to a lumpy curd.

The most favourable temperature for the rennet to set the milk was 20°—22° C. Lower temperatures resulted in slow and irregular drainage of whey from the resulting curd. Higher temperatures caused granular, sour curds.

The following table, by Marquardt, shows the influence of the composition of milk and cream on the cheese made therefrom :—

TABLE 53. INFLUENCE OF THE COMPOSITION OF MILK AND CREAM ON CHEESE.

Factors Studied.	Per Cent. Milk Fat in Cream.							
	6·82.	10·01.	11·75.	13·26.	14·12.	17·45.	17·50.	26·50.
Per cent. solids in cream . .	20·04	19·46	—	22·54	22·98	25·46	—	—
Per cent. solids in curd . .	48·37	—	47·75	44·83	—	—	—	—
Per cent. fat in whey . . .	0·29	0·29	0·13	0·15	0·22	0·27	0·15	1·19
Per cent. solids in whey . .	7·32	7·70	6·94	7·20	7·67	7·80	7·20	8·23
Cheese texture (Av.) . .	Granular.	Slightly granular.	Slightly granular.	Smooth, creamy.	Smooth, creamy.	Very smooth.	Very smooth.	Very smooth.

The relation between the fat content of the cream to the size of the fat globules, and the size and number of the clusters of fat globules to the texture of the cheese was found to be thus :—

TABLE 54. RELATION BETWEEN DISPERSION OF FAT AND CHEESE TEXTURE.

Factors Studies.	Percentage of Fat in Cream.		
	8·42.	13·69.	17·72.
Homogenisation pressure . .	2,500	2,500	2,500
Average size of fat globules .	1·68	1·87	2·05
Number of fat clumps per 100 single fat globules counted .	44	50	63
Average size of fat clumps [μ] .	(3·4 × 4·9)	(5 × 6·7)	(7·6 × 8·9)
Cheese texture	Coarse.	Smooth.	Very smooth.

When the cream contained 15 per cent. or more of fat, adequate drainage of the curd was only possible when the curd was heated to about 35° C. for 2—4 hours. If a bacterial starter had been used, *i.e.*, acidity developed, the temperature had to be as high as 43° C. in heating the curd.

Marquardt's paper contains much of interest for the colloid chemist, but, of course, is restricted to one special type of cheese. Detailed work is required on other and widely different cheeses.

The addition of calcium chloride to milk for cheese-making has long been a practice. Klein and Kirsten [1] added $CaCl_2$ to pasteurised milk in preparing Limburger and other soft cheeses. Lindet [2] proposed that 1 gr. of $CaCl_2$ be added to each 1 litre of milk. He found that coagulation by rennet was accelerated, a firmer clot resulting in a curd, which drained freely. Similarly, Vaillant [3] found that 0·1 per cent. of $CaCl_2$ in the milk increased the rate of coagulation and gave a slightly better yield of Maroille cheese. The whey was much clearer than usual.

Platon [4] found that the addition of up to 0·2 gr. anhydrous $CaCl_2$ per litre of milk increased the speed of rennet coagulation and reduced the time of working in the vat. Amounts of 0·5—1·0 gr. $CaCl_2$ per litre were unfavourable, the coagulation being too rapid and the whey excessive. The fat content of this whey is less than in the ordinary whey ; thus there is an increase in the content of dry substance in the cheese. By the addition of 0·2 gr. $CaCl_2$ per litre of milk, the amount of rennet could be reduced to one-eighth

[1] *Milch-Ztg.*, **27**, 785 (1898). Cf. Sammis and Bruhn, U.S. Dept. Agr., *Bull.*, **165** (1913).
[2] *Lait*, **4**, 2 (1924) ; *Bull. soc. chim.*, **4**, 929 (1913).
[3] *Lait*, **4**, 7 (1924).
[4] *Medd. Centralanstalt. forsöksväsendet jordbruks.*, No. **32** (1927).

of the normal amount used, without increasing the nitrogen content of the whey. Price [1] confirmed this observation.

During the ripening of cheese in storage, there occurs an increase in the water-soluble nitrogenous constituents, the paracasein breaking down into several less complex compounds, under the action of lactic acid, rennin, galactase and micro-organisms.

The following table by Van Slyke and Price [2] illustrates the degradation involved, each analysis being the average of results obtained with twenty-four different cheeses.

TABLE 55. EFFECT OF AGE ON CHEESE.

Age of Cheese.	Nitrogen, expressed as per cent. Nitrogen in Cheese, in form of :—				
	Mono-calcium paracaseinate.	Water-soluble derivatives from Paracaseinate.	Caseoses and Peptones	Amino Acids.	Ammonia.
Months.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1·5	20·18	21·44	7·00	9·90	1·56
3	27·26	31·00	9·20	14·35	2·45
6	27·55	36·15	9·15	20·00	3·52
9	24·14	43·45	8·15	26·50	4·74
12	19·04	44·75	8·10	28·40	5·41
18	12·65	47·25	6·45	36·15	6·62

Regarding the influence of temperature on ripening, Van Slyke and Price find that for each 1° F. rise in temperature over the range 32° F. to 70° F. there is an increase of 0·5 per cent. in the water-soluble compounds, amino acids and ammonia forming more rapidly at the higher temperatures.

The more the moisture content of a cheese the greater is the break-down of the casein-complex. The amount of rennet used in the initial clotting of the milk also influences the subsequent protein degradation, the more the rennet used the greater the amounts of water-soluble protein derivatives in a given time of ripening.[3] Van Dam [4] observed that the pH was a factor in this respect, the break-down of the paracasein by rennin being proportional to the hydrogen-ion concentration. The phenomenon, as due to rennin, does not occur in cheese, which has been made from milk and curd devoid of added acid or acid salt.

Van Slyke and Bosworth [5] have shown that in the ripening of

[1] *J. Dairy Sci.*, **10,** 373 (1927).
[2] " Cheese " (New York, 1927), p. 278. See also the tables collected by Rahn and Sharp, " Physik der Milchwirtschaft " (Berlin, 1928), p. 224 *seq.*
[3] Van Slyke, N.Y. (Geneva) Agr. Expt. Sta., *Ann. Rept.*, No. **12,** 276 (1893).
[4] *Z. physiol. Chem.*, **61,** 147 (1909).
[5] N.Y. (Geneva) Agr. Expt. Sta., *Tech. Bull.*, **4** (1907).

American cheddar cheese, the lactic acid formed by the action of micro-organisms on the lactose in the milk, interacts with calcium paracaseinate (cheese curd) to form monocalcium paracaseinate and calcium lactate. When the cheese is first put into the press its lactose content is from 1—2 per cent., but after 2 weeks it is *nil*. Monocalcium paracaseinate is soluble in 5 per cent. sodium chloride solution, a fact used by these authors as an index of the changes in the cheese curd. Thus [1] :—

	Protein Soluble in Brine.	Protein Soluble in Water.
When curd was cut . . .	3·13 per cent.	—
When whey was removed . .	4·50 ,,	—
When curd was put in press . .	30·15 ,,	3·77 per cent.
2 hours later	46·46 ,,	4·25 ,,
9½ hours later	96·06 ,,	6·48 ,,

It was observed that the formation of the brine-soluble monocalcium paracaseinate is a necessary preliminary to cheese ripening. Such formation is very rapid during the cheddaring operation. During ripening, monocalcium paracaseinate is changed into an insoluble form, at first rapidly, later more gradually for months. The insoluble form (uncombined paracasein) is degraded into water-soluble protein derivatives, the bio-chemistry and colloid chemistry of the changes involved still being open to research.

Salt slows down the ripening of cheese and the formation of water-soluble nitrogenous bodies, particularly the amino acids and ammonia. No doubt this is to be connected with the action of salt in reducing the moisture content of cheese and in restraining enzyme action.

Instead of rennet, pepsin [2] may be used to curdle the milk for cheese-making, except in the case of sweet curd cheese, such as Swiss, Brick or Limburger, since these are produced from milk of low acidity. Merker [3] found that coagulation with pepsin was to a limited extent accelerated by acid, whilst alkali retarded or inhibited the clotting. The time of clotting could be considerably reduced by adding $CaCl_2$ or $CaHPO_2$ to the milk, *e.g.*, milk curdled by pepsin in 30 minutes, could be curdled in 7 minutes by the previous addition of 0·04 per cent. of $CaCl_2$.

[1] Van Slyke and Price, " Cheese," p. 289.

[2] Graber, *Ind. Eng. Chem.*, **9,** 1125 (1917) ; Merker, *J. Dairy Sci.*, **2,** 482 (1919) ; Lucas, Oregon Agr. Expt. Sta., *Bull.*, **155** (1918) ; Fascetti, *Staz. sper. agr. ital.*, **49,** 13 (1916) ; Barr, *Agr. Gaz. Canada*, **4,** 660 (1917) ; Steuart, *J. Board Agr.*, **24,** 57 (1917) ; Bodansky, *J. Biol. Chem.*, **61,** 365 (1924) ; *Biochem. Z.*, **105,** 60 (1920) ; Stevenson, *J. Agr. (New Zealand)*, **14,** 32 (1917) ; Fenger, *J. Am. Chem. Soc.*, **45,** 249 (1923) ; Akiba, *Keio Ig., Tokyo*, **2,** 1387 (1922).

[3] *J. Dairy Sci.*, **2,** 482 (1919).

Cheese, *e.g.*, pot-cheese, may also be made from milk which has been curdled by acid only, cutting the curd when the acid content reaches 0·55 to 0·60 per cent. as lactic acid. Such curd lacks elasticity, being coarse-grained and sticky.

A problem of considerable importance in cheese-making concerns the rate of diffusion of salt from the surface to the interior. Orla-Jensen [1] points out that the external portions of Emmenthal cheese are richer in salt than the interior, hence the fewer holes in the exterior. The only extensive investigation on the matter is that

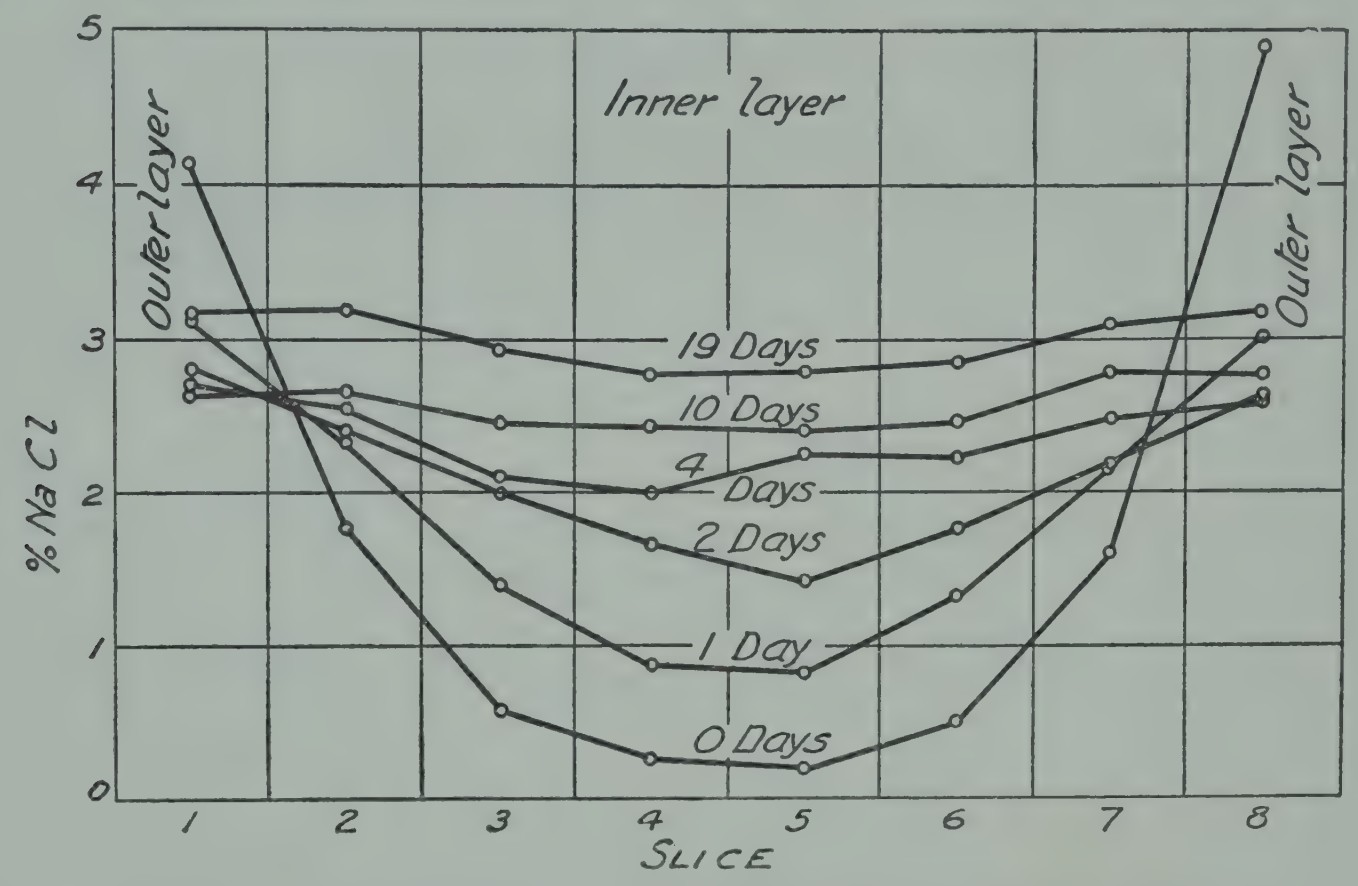


Fig. 30.—Salt diffusion in Limburger cheese in relation to time.

due to Mrozek.[2] Two Limburger cheeses, half fat and full fat cheeses respectively, were placed as soon as made in 15 per cent. sodium chloride solution for 24 hours. The cheeses were then removed from the brine and analysed at intervals for NaCl content in different layers, first removing the external layers 2 cm. deep, and then cutting the cheese into 8 discs. Discs 1 and 8 are thus the immediate external faces, discs 4 and 5 are from the centre of the cheese.

The results are expressed graphically [3] in Fig. 30.

The gradual levelling-up of the salt content throughout the mass is almost completed after 4 days ; beyond that time the gradual drying of the cheese must be taken into account. There is practically no difference in the results whether the cheese be half or full-fat cheese.

Distinct from the salt diffusion, there is osmotic movement of water. Mrozek gives the following typical data :—

[1] *Landw. Jahrb. Schweiz.*, **20,** 437 (1906).
[2] *Milchwirtschaft. Forsch.*, **4,** 391 (1927).
[3] *Vide* Rahn and Sharp, " Physik der Milchwirtschaft," p. 234.

Water Exchange in Salted Cheese (parts water per 100 parts Salt-free Dried Cheese).

Layer Analysed.	Days Elapsed after Salting.							
	0	1	2	4	6	10	15	19
External . .	116·75	122·59	112·47	101·89	102·52	105·64	90·18	92·36
2nd section .	126·91	130·32	118·72	107·16	106·87	105·76	96·12	90·96
3rd section .	125·46	120·24	113·52	102·71	104·61	99·75	92·41	86·97
Internal . .	121·71	117·96	109·77	100·55	101·44	96·86	91·47	84·26

The second section of the cheese, *i.e.*, 0·5—1 cm. below the external surface is highest in water content even up to 15 days. Mrozek believes this is due, not to osmosis, but to a change in the colloidal character of the cheese on ageing, such change leading to increased swelling pressure in the outermost layer, *i.e.*, where ripening is most advanced.

The influence of salt on the size and distribution of the fat globules in cheese calls for investigation, particularly in the softer varieties. Again, the pH value of cheeses in relation to ripening and to taste deserves study and an investigation by Schultz [1] should be recorded in this connection. He found that Tilsiter cheese on storing showed a diminishing acidity of the rind, until in quite old cheese it approached pH 7. That flavour is intimately bound up with the changes that involve pH changes is shown by the fact that the centre of a mild ripe Tilsiter cheese had a pH range of 5·2—5·6, whereas the taste was bitter for the pH range 5·7—5·82.

ICE CREAM

Ice cream manufacture provides some interesting physico-chemical problems, in particular relating to the use of protective colloids and to the function of the homogeniser.

A good account of the manufacture of ice cream (in America) has been presented by Reid.[2] Ice cream is prepared from a mixture of dairy products such as milk, butter, skim milk, condensed milk, milk powder, etc., as well as other substances, such as sugar, honey, eggs, fruit, chocolate, flavours, and protective colloids like gelatin and gum tragacanth. The method employed is to pasteurise the mix at 63° C. and then homogenise, usually at a pressure of 2,500 lbs. per square inch. After cooling to 10° C. the mix is passed to ageing vats for 12—24 hours at 1°—2° C. Freezing [3] is then conducted at — 4° C. with violent agitation and beating, and the mass is now hardened by standing for 24 hours at —4° C.

Ice cream analyses vary greatly owing to the commercial variety

[1] *Molkerei-Ztg.* (*Hildesheim*), **44,** 527 (1930).

[2] *Fourth Intern. Cong. Refrig.* (London, 1924), 928 ; Davel, Union S. Afr. Dept. Agr., *Bull.* **53,** 7—30 (1929).

[3] Freezing data for various mixes are given by Dahle, *Ice Cream Trade J.*, **22,** 63 (1926).

of the mix employed. Williams [1] gives the following approximate ranges :—

	Per cent.
Fat	8—18
Sugar	13—17
Milk solids-not-fat	6—12
Gelatin	0·25—0·5

According to Alexander [2] : " It is a well-known fact to practical ice cream makers and amply proven by experience, that ice cream made without eggs, gelatin or some similar colloidal ingredient, is gritty, grainy, or sandy, or else soon becomes so on standing, whereas ice cream made with small quantities of colloids possesses that rich, mellow, velvety texture, so much in demand."

The protecting colloids inhibit the growth of ice crystals. An ice cream having been whipped during freezing, contains numerous small ice crystals which tend, on standing, to coalesce to form coarser crystals. Such coalescence is retarded by the colloids present and a smoother product results. Zoller [3] has investigated the separation of ice on ice cream, using a rotating thermo-couple attached to a horizontal ice cream freezer. It was found that 0·5 per cent. of gelatin reduced super-cooling in the freezing process, and thus the ice crystals separated rapidly as a finely-divided condition. Glucose syrups also reduce super-cooling.[4]

According to Frohring [5] milk protein acts as an emulsifying agent for the fat and produces smooth texture.

An undesirable feature in ice cream is " sandiness " or grittiness, this resulting from the presence of undissolved lactose.[6] Hence it is important to regulate the lactose content below the saturation point approximately 7 per cent.[7] Glucose, corn syrup, gelatin and milk serum solids seem to offer no protection against lactose crystallisation. Palmer and Dahle,[8] and Palmer [9] studying the crystallisation of lactose in frozen solution at 0° C. found no protective action with colloids and acids in amounts normal to ice cream.

Zoller and Williams [10] support the view that lactose causes " sandiness." They isolated such sandy crystals, which analysed 99·86 per cent. lactose. The form in ice cream is the normal crystal,

[1] " Fundamentals of Dairy Science " (New York, 1928), p. 229. See also : Gronover and Tiemann, *Z. Untersuch. Lebensm.*, **54,** 183 (1927) ; Buttenberg, *ibid.*, **54,** 186 (1927) ; Krupse, *ibid.*, **54,** 191 (1927). American legal standards are listed by Hunziker, " The Butter Industry " (Illinois, 1927), p. 554.

[2] *J. Soc. Chem. Ind.*, **28,** 284 (1900) ; cf. *Kolloid-Z.*, **4,** 86 (1909).

[3] *Ice Cream Trade J.*, **17** (8), 40 ; (9), 45 ; (10), 50 (1921).

[4] Cf. Dahle, *ibid.*, **22,** 63 (1926).

[5] *Ibid.*, **19,** 63 (1923).

[6] For a review of the literature on this see Dahle, *Ice Cream Trade J.*, **19,** 55 (1923) ; Troy and Sharp, *J. Dairy Sci.*, **13,** 140 (1930), which has a bibliography of thirty-one references.

[7] Reid, *Ice Cream Trade J.*, **26,** 60 (1930).

[8] Minnesota Agr. Expt. Sta., *Ann. Rept.* (1922), p. 41.

[9] *Ind. Eng. Chem.*, **16,** 634 (1924).

[10] *J. Agr. Research*, **21,** 791 (1921).

which gives rugged or maize-shaped crystals. Determinations of the velocities of transformation of α—β lactose over the pH range 2—10 and temperature range 0°—50° C. have been made by Parisi.[1] According to the experiments of Troy and Sharp [2] the α-lactose hydrate can be prevented from crystallising out if the water be rapidly frozen out of ice cream, the lactose solution being thereby concentrated to a region where the crystallisation of the α-lactose hydrate does not occur. This agrees with the recent findings of Williams and Peters [3] that high viscosity (hard frozen) modifies the crystal formation, if any, to yield an extremely fine sand.

An interesting paper on " The Effects of Binders upon the Melting and Hardness of the Ice Cream," by Holdaway and Reynolds,[4] reports that as the per cent. of fat in plain ice cream increases, the cream becomes softer, and if too much fat is present a soft, fluffy product results owing to whipping. Ice cream made from 8 per cent. cream was no harder than from 19 per cent. cream, whilst 30 per cent. plain cream was much softer than either. The fat, however, raises the melting point. On the other hand, Nelson and Reid [5] find no direct relation between the fat content and hardness, a conclusion also reached by Fisher.

If gelatin be added a harder ice cream results with a higher melting point. The hardest and most heat-resisting ice cream is given by a medium per cent. of fat and a large amount of gelatin. Gum tragacanth may be used, in which case the ice cream produced is harder than usual (plain) but softer than when gelatin is used. If too much gum is used, a very slimy cream results ; in any case, it is inferior to gelatin.

Turnbow and Raffetto,[6] dealing with the influence of gelatin on the viscosity of ice cream, show that increasing the gelatin concentration greatly increases the viscosity, but not in corresponding ratio. Thus :—

Time (Hours).	Gelatin (Per cent.).	Viscosity (Poises).
24	0·2	0·646
24	0·3	1·020
24	0·4	4·280
24	0·5	12·280
24	0·6	28·920
48	0·3	1·42
48	0·4	5·64
48	0·5	18·60
48	0·6	Very thick.

[1] *Giorn. chem. ind. applicata*, **12,** 225 (1930).
[2] *J. Dairy Sci.*, **13,** 140 (1930).
[3] *Ibid.*, **13,** 471 (1930).
[4] Virginia Agr. Expt. Sta., *Bull.*, **211,** 3 (1916).
[5] Missouri Agr. Expt. Sta., *Res. Bull.*, **70,** 24 pp. (1924).
[6] " Ice Cream " (New York, 1928), p. 124.

Some manufacturers use starch [1] to improve ice cream texture, but although a better product than plain ice cream results, the texture is more grainy than when gelatin or gum is employed. At first sight, one would be tempted to connect this with the fact that the protective action of starch is poor, its gold number being up to 25, whilst that of gelatin is 0·005 to 0·01. However, there seems to be no relation between the gold number of a gelatin and its value in an ice cream mix.[2]

Eggs are good " fillers " or " binders," since egg albumin (with a gold number of 0·15 to 0·25) is a protective colloid. Egg *yolk* improves the whipping quality of the mix and facilitates freezing, but has no influence on texture.[3] Dried egg yolk is less effective, whilst dried egg albumin is actually detrimental.[4]

A detailed study of the factors influencing ice cream texture has been made by Dahlberg.[5] He found that the air cells in ice cream varied considerably in size, the average diameter in the case of homogenised product being 60μ. The diameters bore no relation to the composition of the mix but were influenced by homogenisation and freezing conditions. Thus :—

Time in Freezer.	Condition of Ice Cream.	Diameter of Air Cells (μ).		
		Min.	Max.	Av.
5 mins.	Starting to whip . .	22	290	124
8 ,,	Whipped sufficiently. .	21	190	89
11 ,,	Properly frozen . .	11	150	62

Excellent photomicrographs illustrate this aspect of the subject. Texture was not seriously influenced by the size of the air cells, a finding contrary to that of Given [6] who found the quality depended on the number and diameter of the air cells.

The effect of the various ingredients was studied. Sugar reduced the amount of ice formed and so improved texture. The water only partially froze out leaving a concentrated (super-saturated) sugar solution, which would not freeze.

Gelatin was found to possess no greater protective action in inhibiting ice growth than casein, and its chief value is believed to be its ability to form a gel in the frozen product, " which, on account

[1] Holdaway and Reynolds, Virginia Agr. Expt. Sta., *Bull.*, **211**, 3 (1916).
[2] Moore, *et al.*, *J. Dairy Sci.*, **8**, 500 (1925); Ferris, *ibid.*, **5**, 555 (1922); Downey, *Am. Food J.*, **19**, 334 (1924); Lehmkuhl, *Ice Cream Trade J.*, **20**, 65 (1922).
[3] Martin and Caulfield, *J. Dairy Sci.*, **12**, 193 (1929); Mueller and Button, *ibid.*, **12**, 320 (1929).
[4] Mueller and Button, *ibid.*, **12**, 331 (1929).
[5] N.Y. (Geneva) Agr. Expt. Sta., *Tech. Bull.*, **111**, 1—42 (1925).
[6] *Ind. Eng. Chem.*, **20**, 966 (1926).

of its stiffness and internal structure, prevents the growth of large ice crystals."

The presence of milk fat favours a smooth texture by mechanically obstructing the growth of ice crystals. A mix containing 30 per cent. milk fat, 0·3 per cent. proteins, and 13 per cent. sugar remained smooth in the ice cream hardening room, whilst a mix containing 30 per cent. milk solids-not-fat and 13 per cent. sugar became coarse, the water freezing into crystals and long spines. Thus the colloid-protective action of the proteins present is slight. "Milk fat and solids-not-fat had a greater combined effect on texture than either alone. When milk fat and solids-not-fat were present in the same mixture, the percentage of gelatin required to form a gel was reduced, indicating that the gelatin was not dispersed in all the water. It was assumed that the water withheld from the gelatin froze to form small ice crystals in ice creams."

The effect of homogenisation was to smooth the texture. Homogenisation so affected the system that less gelatin was necessary to form a gel in ice cream mixtures.

Homogenisation of Ice Cream. During the pasteurisation of the ice cream mix the viscosity is reduced and the subsequent homogenisation is intended to restore the viscosity (see p. 236). The emulsion now assumes a finer texture and a smoother, rich appearance. Homogenisation also decreases the danger of churning fat (separation of butter-fat) in the freezer.[1]

In an exhaustive study of the homogenisation process, Reid and Moseley [2] show that the restoration of viscosity is due to (*a*) adsorption at the enlarged fat-liquid boundary, (*b*) clustering of the fat globules. The surface tension of the mix increases. Similarly, Sherwood and Smallfield [3] found that, in general, the viscosity of an ice cream mix increases on ageing, but the rate of increase is intensified if the mix be homogenised. The pasteurised mix shows an even distribution of fat globules, whereas in the homogenised mix the globules were about one-tenth the original size and clustered together, from three or four to several hundred globules, forming clusters of various sizes. Agitation breaks down these clusters with a consequent rapid drop in viscosity.

Hening [4] studied the effect of homogenising mixes wherein the gelatin was added prior to, or after, homogenisation. It was found that a higher viscosity followed the addition of gelatin after homogenisation. When the gelatin was present during homogenisation

[1] Mortensen, *Ice Cream Trade J.*, **20,** (4), 74 (1924) gives photomicrographs in this connection. See also Reed and Garrison, Missouri Agr. Expt. Sta., *Res. Bull.*, **128,** 1—41 (1929).

[2] Missouri Agr. Expt. Sta., *Res. Bull.*, **91,** 1—25 (1926).

[3] *J. Dairy Sci.*, **9,** 68 (1926).

[4] *Ibid.*, **11,** 299 (1928).

the size of the fat clusters increased slightly. The freezing and whipping properties were not influenced by the fact of the prior nor later addition of the gelatin.

Homogenisation with sugar gave consistently higher viscosities than when the sugar was added after homogenisation. A detailed study was made of the degree of dispersion of the fat and of the number and size of fat globule clusters in the mixes homogenised with and without sugar. There was no change in the size of the individual fat globules due to the presence of sugar during homogenisation, but the number of fat clusters and their size increased. "Measurement of 1,235 individual globules of homogenised mixes without sugar with the single-stage valve, gave an average size of 1·55 microns as compared with a size of 1·72 microns obtained for 1,327 measurements of fat globules in the mix homogenised with sugar. The size of the fat cluster increased 1·69 × 1·11 microns when the mix was homogenised with sugar." Freezing the mix reduced the size of the fat clusters about 40 per cent.

An interesting table showing the effect of homogenisation on surface tension and viscosity of ice cream mix has been presented by Turnbow and Nielson.[1] The mix contained 11 per cent. fat, 10·5 per cent. milk solids-not-fat, 15·5 per cent. sugar and 0·35 per cent. gelatin. Pasteurisation was conducted at 63° C. and homogenisation at the same temperature at a pressure of approximately 1,133 kg. per sq. cm. Viscosity measurements were made at intervals after the mix left the cooler, using a MacMichael viscometer. The fresh mix (once homogenised) had a viscosity of 0·7921 poise, but after 5 hours' ageing this rose to 3·3884 poises. If now this sample was placed on a shaking machine the viscosity could be reduced to 0·7481 poise, *i.e.*, to approximately the value for the fresh mix (Table 56).

Reid and Scism [2] have pointed out that whilst homogenisation increases viscosity of an ice cream mix and this viscosity increases if the homogenised mix be passed through an emulsifier, passage a second or third time through an homogeniser reduces the viscosity. Similarly, Hening [3] observed this reduction in viscosity and connected it with the decrease in size of the fat globule clusters, the use of a second-stage homogenising valve effecting a 30 per cent. reduction in the size of the clusters. Some of his results are given on p. 267.

The use of a two-stage valve [4] is now recognised as giving a smoother texture to ice cream due to the re-dispersion of the fat

[1] *Ind. Eng. Chem.*, **20,** 377 (1928).
[2] Missouri Agr. Expt. Sta., *Res. Bull.*, **82,** 1—22 (1925).
[3] *J. Dairy Sci.*, **11,** 299 (1928).
[4] Cf. Snow, U.S. Pat. 1,690,799 (1928).

Viscosity of Ice Cream Mix in Relation to Homogenisation.

	Homogenised without Sugar.		Homogenised with Sugar.	
	Single-Stage Valve.	Two-Stage Valve.	Single-Stage Valve.	Two-Stage Valve.
Experiment 1 . .	2,800	2,190	3,600	1,800
Experiment 2 . .	2,720	870	5,360	4,900
Experiment 3 . .	1,570	990	2,080	1,300
Experiment 4 . .	1,530	620	4,480	2,880

MacMichael Viscometer : Data expressed as centipoises.

clusters. Photomicrographs showing the butter-fat clustering as a result of processing with a single-stage valve and the freedom from clusters when using a two-stage valve, are given by Turnbow and

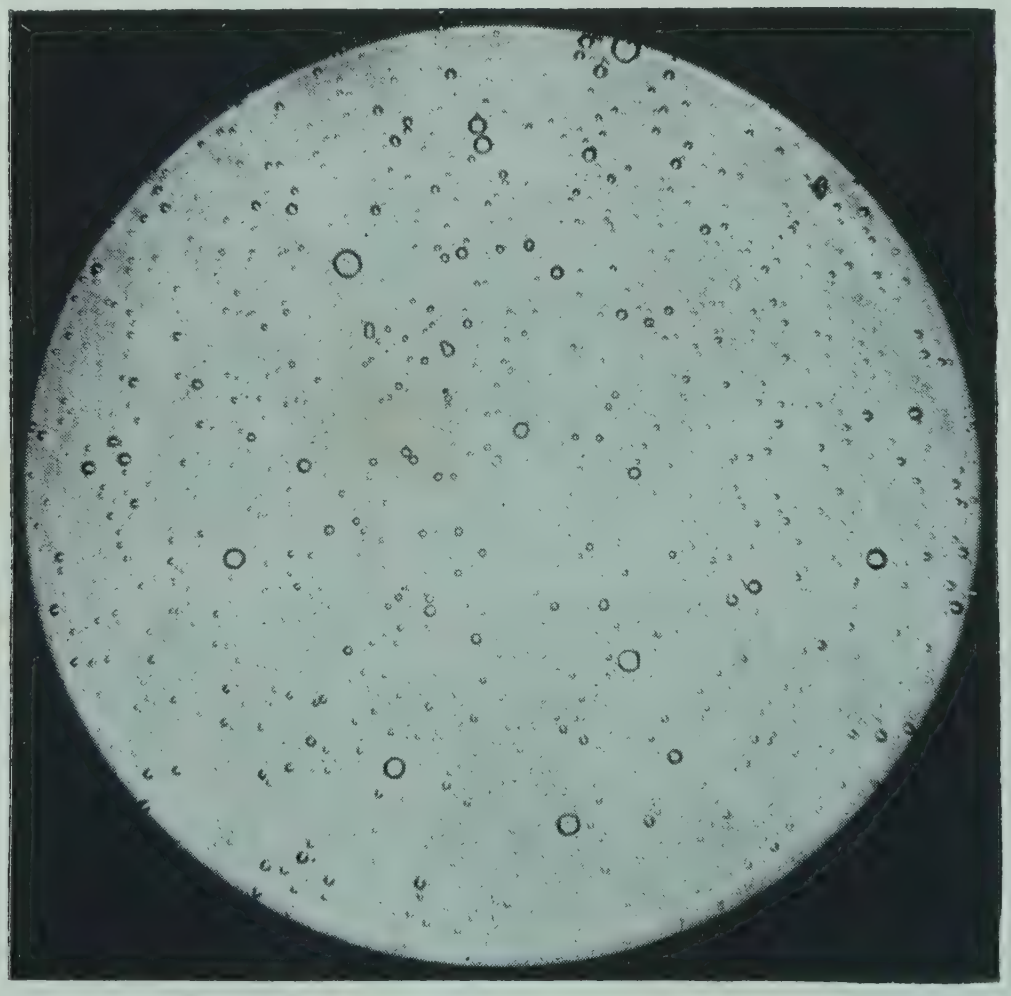


FIG. 31.—Unviscolised ice cream mix.

Nielson. It is advisable to have high pressure on the first valve but only 500 lbs. pressure on the second.[1] Figs. 31, 32, 33 reproduce similar photomicrographs by Dahle.

[1] Turnbow and Raffetto, " Ice Cream " (New York, 1928), p. 178.

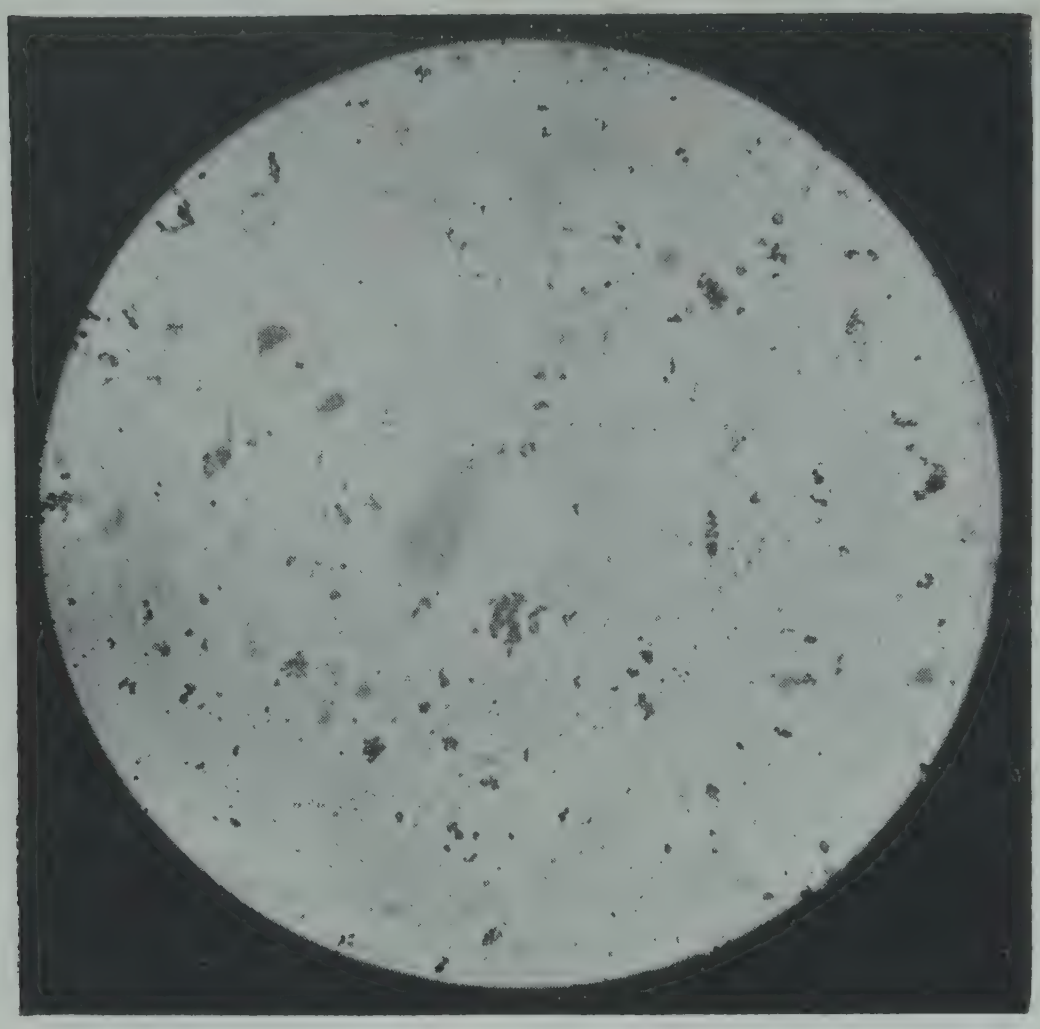


FIG. 32.—Ice cream mix viscolised at 2,500 lbs./sq. in., using a regular valve.

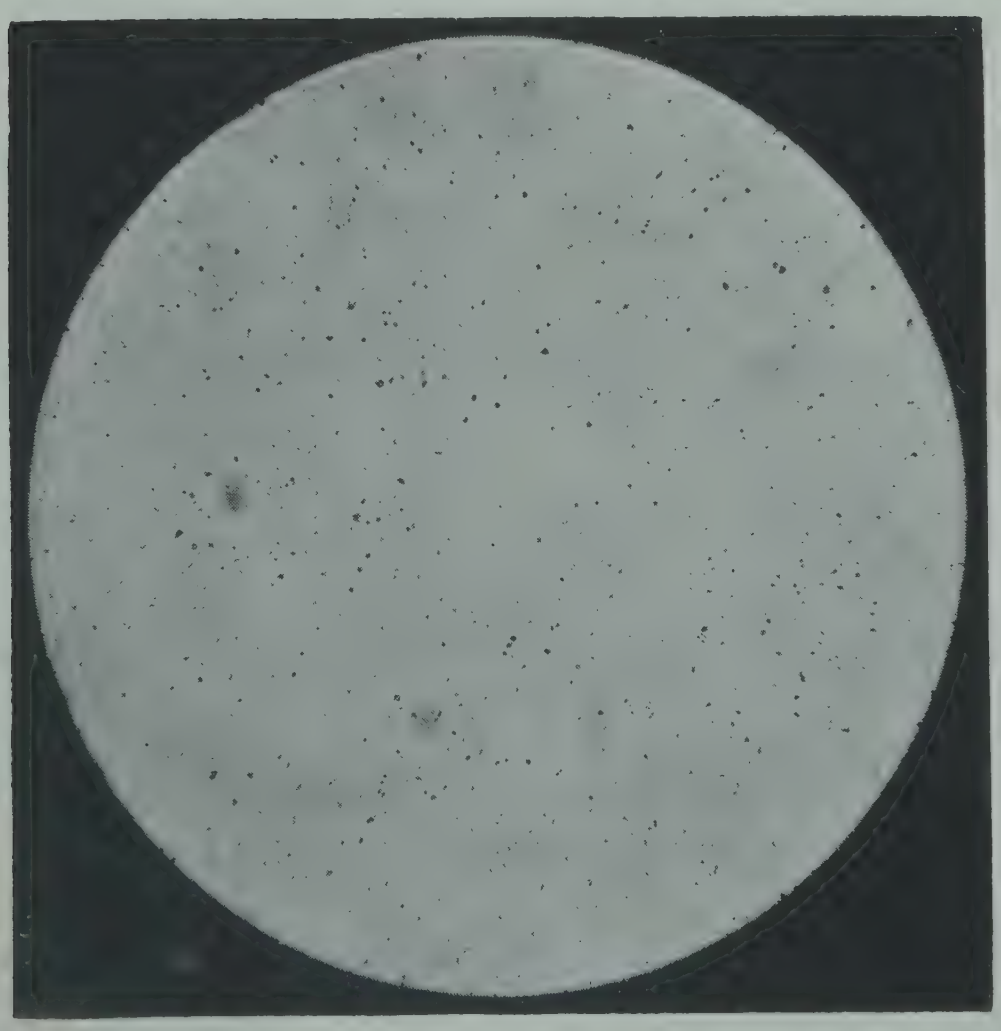


FIG. 33.—Ice cream mix viscolised at 2,500 lbs./sq. in., with No. 3 breaker ring.

Table 56. Viscosity and Surface Tension of Ice Cream Mix Repeatedly Homogenised.

	Fresh.			Aged 5 Hours.				Aged 24 Hours.			Aged 48 Hours.			Aged 72 Hours.			
	Temp.	Shaken.	Viscosity.	Temp.	Shaken.	Viscosity.	S. Tension.	Temp.	Shaken.	Viscosity.	Temp.	Shaken.	Viscosity.	Temp.	Shaken.	Viscosity.	S. Tension.
	°C.		Poises.	°C.		Poises.	Dynes/cm.	°C.		Poises.	°C.		Poises.	°C.		Poises.	Dynes/cm.
Once .	16·7	—	0·7921	16·7	—	3·8884	63·70	15·6	—	5·6327	15·6	—	6·9529	15·6	—	10·0333	63·68
				16·7	1,400	0·7481	63·70	15·6	1,400	0·7481	15·6	1,200	0·7481	15·6	1,400	0·7481	63·76
Twice .	16·7	—	0·7481	16·7	—	1·5842	63·96	15·6	—	2·5523	15·6	—	2·9923	15·6	—	4·5766	64·11
				16·7	1,400	0·6161	63·99	15·6	1,400	0·5721	15·6	1,200	0·5721	15·6	1,400	0·6601	64·16
Thrice .	14·4	—	0·3961	16·7	—	0·9241	64·08	15·6	—	2·6403	15·6	—	3·0364	15·6	—	2·9924	64·16
				16·7	1400	0·4841	64·19	15·6	1,400	0·4841	15·6	1,200	0·4841	15·6	1,400	0·4841	64·19

WATER ICES AND SHERBETS

The semi-frozen product prepared from water, sugar and fruit juice is termed a water ice, whilst sherbet is a similar product containing milk or milk products. In the manufacture of these products the unfrozen syrup has a tendency to drain out during storage in the hardening room even at —17·7° C. The product then has the defect of being very hard in the upper part of the container and very soft, mushy and sweet in the lower part. Stabilisers, such as gum tragacanth, have been employed to prevent syrup drainage. An extensive study of the influence of colloid stabilisers has been made by Dahlberg.[1] He observed that gum tragacanth, while preventing most of the syrup from draining, gave an undesirable crumbly texture. Gelatin forms a jelly in the water ices or sherbets and gives a smooth product, devoid of crumbliness or syrup leak ; its defect is the undesirable amount of air incorporated during freezing. Agar (0·2 per cent.) which proved as good as gelatin, made whipping difficult, but this was overcome very satisfactorily by using it in conjunction with milk proteins, gelatin or gum tragacanth.

ARTIFICIAL MILK

Of late years artificial milk has been prepared and numerous patents secured in this connection. It is simple enough to prepare a solution containing the inorganic constituents of milk, but the organic (colloidal) constituents present a more difficult problem.

Certain nuts and beans contain proteid substances closely akin to gluten or casein in properties, *e.g.*, soya beans contain about 37 per cent. of such proteid matter, which can be brought into colloidal solution by boiling with alkali or salts which react alkaline, *e.g.*, sodium phosphate. Thus von Riegler [2] prepared an artificial milk by dissolving gluten in caustic potash. It is usual, however, to emulsify up to about 3 per cent. of oil in the colloidal solution and then to add appropriate quantities of lactose, lactic acid, citric acid, etc., to render the similarity to milk more complete. Other ingredients which find their way into artificial milks are dextrin, malt extract, sugar, honey, egg-yolk and salt.

Soya bean milk has been recommended for children suffering from gastro-intestinal diseases, as it is very easily tolerated, sometimes more so than human milk.[3] Blunck [4] describes such a milk prepared by the fine grinding of 100 gr. of soya beans in 1 litre of water. The

[1] N.Y. (Geneva) Agr. Expt. Sta., *Bull.*, **536** (1926).

[2] French Pat., 461,131 (1913).

[3] Muggie and Gasca, *Gazz. Ospedali clin.*, **42,** 356 (1921). *Vide* Vandevelde, *Ann. bull. soc. roy. méd. Gand.* (1919), p. 66 ; Tso, Yee and Chen, *Chinese J. Physiol.*, **2,** 409 (1928).

[4] *Chem.-Ztg.*, **45,** 1166 (1921).

resulting milk analysed : water 90, protein 2, fat 4·5, carbohydrate 2·5, ash 1 per cent.

Analyses of soya bean milks have been reported by various authorities. Adolph and Kiang [1] state that a sample of Chinese soy-milk contained 4·22 per cent. protein, 1·8 per cent. fat, and 0·4 per cent. ash. An analysis by Remy [2] gives 88·93 per cent. water, 11·07 per cent. dry matter, 3·06 per cent. fat, 2·96 per cent. protein, 0·57 per cent. starch, 2·48 per cent. glucose, and 0·63 per cent. ash. Soya bean milk has been discussed by Kiesselbach [3] and Nakayasu,[4] vegetable milks by Chapin,[5] and special protein milks by Bartley [6] and d'Espine.[7]

Palmer [8] has described the preparation of artificial acacia milk. Fifteen grains powdered U.S.P. acacia were ground with 20—25 c.c. distilled water until the gum was thoroughly hydrated. Twenty grains melted butterfat was emulsified, adding in 2—3 c.c. lots with thorough stirring. Dilution with warm water followed to a volume of 500 c.c. The resulting "milk" contained 4·0 per cent. fat, and the reaction was pH = 4·96. Artificial milks prepared from almonds and Brazil nuts have been used by Fischer [9] with excellent results. He notes the very fine curd given on acid coagulation of the milks.

The patent literature reveals some interesting points on the colloid aspects of synthetic milks. The principal patents relating to soy bean milk are :—

Berczeller, L. and Graham, R.	Brit. Pats. 157351, 157352 (1921).
Burdick, A. S. and Nielsen, C.	U.S. Pats. 1273144, 1273145 (1918).
Erslev, K.	U.S. Pat. 1297668 (1919).
Melhuish, W. J. . . .	Brit. Pats. 9626 (1915), 118535 (1917). Norw. Pat. 27895 (1917). U.S. Pat. 1243855 (1917).
Moses, A. B. . . .	U.S. Pats. 1281411 (1918), 1332562 (1920).
Thevenot, G. D.. . .	U.S. Pats. 1359633 (1920), 1556977 (1925).
Yamamoto, Y. . . .	Jap. Pat. 40711 (1921).

[1] *Nat. Med. J. China*, **6,** 40 (1920).
[2] *Z. Untersuch. Lebensm.*, **43,** 380 (1922) ; cf. Zlataroff and Trifonow, *ibid.*, **44,** 214 (1922).
[3] Nebraska Agr. Expt. Sta., *Bull.*, **166,** 1—16 (1918).
[4] *J. Pharm. Soc. Japan*, (1921), No. **476,** p. 880. See also Tso, *Chinese J. Physiol.*, **2,** 33 (1928).
[5] *Arch. Pediatrics*, **35,** 365 (1918).
[6] *Ibid.*, **39,** 741 (1922).
[7] *Intern, J. Pub. Health*, **1,** 34 (1920).
[8] *J. Am. Chem. Soc.*, **44,** 1529 (1922).
[9] *Arch. Verdauungs-Krankh.*, **20,** 13 (1914).

Important patents relating to artificial milk of various compositions have been granted as follows :—

Beckman, H. C., and Dyck, C. E.	U.S. Pats. 1190369, 1191388 (1916), 1216052 (1917).
Bosworth, A. W.	U. S. Pat. 1450836 (1923).
Dunham, H. V.	Brit. Pat. 180018 (1921).
Erslev, K.	Brit. Pat. 121133 (1918).
Gates, R. W. B. S. J.	Brit. Pat. 215039 (1922).
Gössel, F.	Holl. Pat. 2122 (1917).
Habbema, H. T.	U.S. Pat. 1403405 (1922).
Hay, J. G.	Brit. Pat. 168276 (1920).
Jurgen, A.	Brit. Pat. 128544 (1919).
Melhuish, W. J.	Brit. Pat. 13903 (1915). U.S. Pat. 1210667 (1917).
North, C. E.	U.S. Pat. 1474843 (1923).
Rigler, G.	German Pat. 291130 (1912). Brit. Pat. 18382 (1913).
Schlinck, J.	Brit. Pat. 104933 (1916). U.S. Pat. 1220010 (1917).
Stevens. C. S., and Baumann, C. A.	Brit. Pat. 125172 (1917).
Townsend, C. S.	Brit. Pat. 160234 (1919).

CHAPTER VIII

COLLOID ASPECTS OF NUTRITIONAL CHEMISTRY

Foods are typically colloidal in their nature and the processes accompanying their breakdown and their assimilation in the body deal with the changes : colloid→molecular condition→colloid.[1]

The main function of the stomach is to bring about a *physical* preparation of food in order that the pancreatic and intestinal juices can complete digestion in the small intestine.[2] Food is finely broken up and intimately mixed with gastric juice by muscular movements (pyloric waves) in the stomach. This results in a thin liquor, *chyme*, which is gradually passed *via* the pyloric sphincter into the duodenum.

Although the mechanism of the regulation of the pyloric sphincter is still obscure, the balance of opinion favours the theory that the state of subdivision of the food plays a part. Maclean [3] states : " There is no doubt that solid objects when pressed against the sphincter tend to keep it closed, while on the other hand, the presence of liquid food in the stomach seems to favour relaxation."

The fact that fine subdivision of food greatly assists digestion [4] is well known in connection with such foods as homogenised milk [5] and ice cream.[6] One reason why goats' milk is so readily digested by infants lies in the fact that the fat globules are much smaller than in cows' or human milk and are therefore more accessible to the lipase of the digestive juices.[7] Again, there seems to be a direct relationship between the size of starch grains and the amount digested,[8] although the quantity of starch fed is also a factor.

Particularly in the case of fats do we find the influence of subdivision important in relation to the case of digestion. Emulsified fats disappear from the stomach much more rapidly than when non-emulsified. For non-emulsified fats the rate of disappearance depends on the melting point, those fats with low melting-point

[1] For an excellent essay on this subject see McCollum in Bogue's " Theory and Application of Colloidal Behaviour " (New York, 1924), Vol. II., p. 683—699.

[2] Maclean " Modern Views on Digestion and Gastric Disease " (London, 1928), p. 6 ; Babkin, *Physiol. Rev.*, **8,** 365—92 (1928).

[3] *Loc. cit.*, p. 8.

[4] Rosenstern and Lauter, *Z. Kinderheilk.*, **32,** 316 (1922).

[5] Washburn and Jones, Vermont Agr. Expt. Sta., *Bull.*, **195,** 6 (1916).

[6] Howell, *Ice Cream Trade J.*, **20** (ii.), 60 (1924).

[7] Schultz and Chandler, *J. Biol. Chem.*, **46,** 133 (1921).

[8] Langworthy and Deuel, *ibid.*, **52,** 251 (1922).

being readily emulsified and showing a higher coefficient of digestion.[1] In illustration of this may be quoted the work of Holmes and Deuel[2] on the digestibility of hydrogenated oils. Taking arachis oil as example, the following figures record the coefficient of digestibility of hydrogenated samples of different melting-points :—

Melting-point (°C.).	Digestibility (per cent.).
37	98·1
39	95·9
43	96·5
50	92·0
52·4	79·0

The cooking of fish in oil retards digestion of the fish owing to the mechanical effect of the oil, decreasing the area of contact between the fish particles and the digestive juices.[3]

By far the major problem of a colloid character, however, concerns the changes taking place in the stomach after milk is drunk, a problem of great importance in pediatrics.

INFANT FEEDING AND THE PHENOMENON OF COLLOIDAL PROTECTION

The normal food for a normal infant is, of course, human milk. In many instances, however, the mother is unable to feed the child by the breast and cows' milk, plain or modified, is employed as a substitute for human milk. Again, in certain infant illnesses specially modified milk diet is indicated. The subject is one of fascinating interest to the colloid chemist, involving considerations of the changes in the milk occurring in the human stomach, these changes being mainly concerned with casein clotting. The familiar problem of the behaviour of protective colloids at once arises.

HUMAN MILK

Numerous analyses of human milk have been reported by Myers,[4] Bell,[5] Bosworth,[6] and others.[7] The following summarises the more

[1] von Fejer, *Biochem. Z.*, **53,** 168 (1914) ; Filinski and Markert, *Compt. rend. soc. biol.*, **96,** 344 (1927).
[2] *Amer. J. Physiol.*, **54,** 487 (1921).
[3] Glassmann, *Z. Untersuch. Lebensm.*, **55,** 231 (1928).
[4] *Brit. J. Dis. Childhood*, **24,** 249 (1927).
[5] *J. Biol. Chem.*, **80,** 239 (1928).
[6] *Ibid.*, **20,** 707 (1915).
[7] Denis and Talbot, *Am. J. Dis. Children*, **18,** 93 (1919) ; Meigs and Marsh. *J. Biol. Chem.*, **16,** 147 (1914) ; Courtney and Brown, *Arch. Dis. Childhood*, **5,** 36, (1930).

important average analyses in contrast to the average analysis of cows' milk (quantities in per cent.) :—

Constituent.	Cows' Milk.	Human Milk.		
		Bosworth.	Myers.	Meigs and Marsh.
Protein . .	2·5—4	1·5	1·58	0·7—1·5
Fat . .	2—4	3·3	3·85	2—4
Lactose . .	3·5—5	6·5	6·0	6—7·5

Elsdon [1] has published the following summary of the analyses of sixty-seven samples of human milks :—

	Average.	Maximum.	Minimum.
Total solids . . .	11·7	14·6	9·6
Solids-not-fat . . .	8·59	9·1	8·0
Fat	3·11	5·9	1·1
Protein	1·19	1·8	0·7
Ash	0·21	0·3	0·2
Lactose	7·18	7·6	6·6

The content of salts averages [2] (in per cent.) : sodium citrate, 0·055 ; potassium citrate, 0·103 ; $CaCl_2$, 0·059 ; KH_2PO_4, 0·069 ; $MgH_4P_2O_8$, 0·027. Mai and Keller [3] propose the differentiation of cows' and human milk based on the ratio of chlorine to lactose, this being $2·23 \pm 0·05$ in the case of cows' milk and only $0·44 \pm 0·015$ in the case of human milk.

As the time interval post partum increases, the average amounts of ash and protein decrease, whilst the fat and lactose increase.[4]

The dialysis of human milk through parchment has been studied by Laxa,[5] who found 75 per cent. of the mineral content was dialysable. Later [6] he was able to dialyse out of 81·5 per cent. of the mineral matter of which two-thirds were water-soluble. His comparison of the ash constituents of human milk and cows' milk is thus : NaCl 2 : 1, K. citrate 3 : 1, K. phosphate 1 : 3, Mg. phosphate 1 : 2, Ca. citrate combined with casein $<$ 2 : 3.

The fat globules in human milk have diameters of the order

[1] *Analyst*, **41,** 74 (1916).
[2] Bosworth, *loc. cit.* See also Telfer, *Glasgow Med. J.*, **113,** 246 (1930).
[3] *Klin. Wochschr.*, **9,** 535 (1930).
[4] Bell, *loc. cit.*
[5] *Lait*, **2,** 428 (1922).
[6] *Casopis lekaru ceskych.*, **66,** 1792 (1927).

30 $\mu.\mu$.[1] The milk reacts practically neutral, its pH value according to Ohi [2] being 6·9—7·1. Davidsohn [3] found the average value to be pH 6·97, ranging from pH 6·6—7·2. On the other hand, slightly higher pH values are given by Clark,[4] *viz.*, pH 7—7·6, average pH 7·22.

COLLOIDAL PROTECTION

Certain colloidal substances, such as gelatin, albumin or sodium oleate favour the stability of colloidal suspensions and act in the opposite direction to the coagulating influence of electrolytes. Thus, colloidal metals are only possible as hydrosols in the presence of a "protecting agent" or "protective colloid." Indeed, the quantitative determination of protective power is based on the capacity of the protecting colloid to inhibit the coagulation of gold hydrosol by an electrolyte.

Zsigmondy [5] proposed the term "gold number" as a measure of protective action. The gold number of a colloid is that weight in milligrammes of the colloid, which just fails to prevent a change in colour from red to violet, when to 10 c.c. of a gold hydrosol (0·0058 per cent. Au) is added 1 c.c. of 10 per cent. NaCl solution.

The following are some typical gold numbers :—

Egg-albumin	0·08—0·10
Globulin	0·02—0·05
Gum arabic	0·5
Gum tragacanth	2·0
Gum tragon	0·15
Irish moss	0·9
Agar-agar	0·5
Gelatin	0·005—0·012
Casein	0·01
Dextrin	6—12
Wheat starch	4—6
Rice starch	9
Maize starch	30·5
Potato starch	25
Sodium oleate	0·04—1
Pectin (citrus)	0·42

In general, the protective colloid is a readily hydrated substance, adsorbable at the interface between the suspended material and the aqueous solution. In this way, some of the characteristic properties

[1] Alexander and Bullowa, *Chem. News*, **101,** 194 (1910); Kreidl and Neumann, *Arch. Physiol.* (*Pflüger's*), **123,** 523 (1908).
[2] *Japan Med. World*, **4,** 44 (1924).
[3] *Z. Kinderheilk.*, **9,** 11 (1913).
[4] "The Determination of Hydrogen Ions" (London, 1928), p. 577.
[5] *Z. anal. Chem.*, **40,** 697 (1901); Schulz and Zsigmondy, *Beitr. chem. physiol. Path.*, **3,** 137 (1903).

of the emulsoid colloid are conferred on the original suspension, and in particular with respect to the influence of added electrolytes.[1]

When two solutions, which on mixing normally yield an insoluble precipitate, contain a protective colloid in sufficient amount, precipitation can be avoided and a colloidal suspension obtained. Thus, Alexander [2] added gelatin to aqueous solutions of sodium phosphate and $CaCl_2$ and obtained a colloidal calcium phosphate, which could be precipitated by both acid and rennin. Emulsification of olive oil in this colloidal system yielded a stable artificial milk.

PROTECTIVE COLLOIDS IN MILK

It has long been known that infants can digest human and asses' milk more easily than cows' milk, and years ago Jacobi [3] reported that "asses' milk has always been recognised as a refuge in digestive disorders, when neither mothers' or cows' milk, or its mixtures, were tolerated." Asses' milk is readily digested and assimilated and is recommended for feeble, diarrheic and athrepsic infants.[4] Reindeer milk serves a similar purpose,[5] whilst goats' milk is also recommended.[6]

The important factor in this connection is that in cows' milk the ratio of lactalbumin to casein is only 0·53 to 3·02, whereas in human milk it is 1·26 to 1·03, and in asses' milk 1·55 to 0·67 (or over double the amount). Of the total protein in cows' milk casein forms nearly 83 per cent., whereas in human milk casein constitutes about 33 per cent. of the total protein.

Human milk is hardly curdled at all by acid or rennin,[7] the reverse being true with cows' milk. We see, therefore, that the ratio of protective colloid (lactalbumin) to the irreversibly coagulative colloid (casein) is a factor of prime importance.[8]

According to Koplik,[9] "cows' milk precipitates or coagulates very early with the aid of acids or salts; women's milk quite late or not at all. Hence in the infant stomach, cows' milk does not take up much acid of the gastric juice and soon coagulates in large masses. Women's milk, on the other hand, takes up a large amount of acid of the gastric juice and coagulates late in small masses. The differences in the modes of coagulation in the two caseins are of great importance in the study of infant feeding."

[1] For detailed discussions see Gortner, "Outlines of Biochemistry" (London, 1929), pp. 191—7; Freundlich, "Colloid and Capillary Chemistry" (London, 1926), pp. 589 *seq.*; Peskov, *J. Russ. Phys. Chem. Soc.*, **49,** 1—63 (1917) on "The Question of Colloidal Protection."

[2] *Ind. Eng. Chem.*, **15,** 284 (1923); *Chem. and Ind.*, **43,** 1256 (1924).

[3] *J. Am. Med. Assoc.*, **51,** 1216 (1908).

[4] Cf. Ribadeau-Dumas and Fouet, *Bull. soc. hyg. aliment.*, **12,** 129 (1924).

[5] Yeppo, *Z. Kinderheilk.*, **43,** 255 (1927).

[6] Cf. Calvin, *Arch. Pediatrics*, **38,** 584 (1921).

[7] Cf. Lesné and Coffin, *Comp. rend. soc. biol.*, **97,** 64 (1927).

[8] Rice, *J. Elisha Mitchell Sci. Soc.*, **43,** 20 (1927); Loebenstein, *Kolloid-Z.* **32,** 264 (1928).

[9] "Diseases of Infancy and Childhood" (1902).

Cows' milk can be made to resemble human or asses' milk in protective qualities by the addition of such colloidal substances as gelatin, gum arabic,[1] cereal gruel or barley water (a starch solution).[2] Aschenheim and Stern [3] investigated the curdling of mixtures of milk and water by rennin in the presence of oatmeal extracts, etc. Milk containing oat jelly gave a curd akin to that from human milk, whilst the addition of lactose minimised the differences in the various curds. A suitable thick cereal feeding [4] is made by adding 3 ounces by volume of flour to 20 ounces of milk, cooking for 15 minutes in a double boiler until the total volume is reduced to 15 ounces. This feeding then contains 5·5 per cent. protein, 4·8 per cent. fat and 16 per cent. carbohydrates.

The coagulation of milk by acid in the presence of six different starches, dextrin, alkali glutinate, egg-white, peptone, gelatin and sodium nucleate was investigated by Tadokoro and Sato,[5] who found the best protective action with sodium nucleate > alkali glutinate > dextrin. Sodium nucleate also delayed coagulation by rennin.

THE CASEIN CURD FROM MILK

Speaking generally, when acid is added to cows' milk, a curd (casein) is precipitated. The quantitative study of this phenomenon, so important in the problem of infant feeding, has been made by Anderson.[6] He found that addition of N/10 HCl to diluted cows' milk caused precipitation of casein, a certain definite quantity of acid giving immediate precipitation with a very rapid (maximum) settling of the curd, such curd being the coarsest at this point. Only slow separation of curd followed the addition of a lesser amount of acid. Should the amount of acid added exceed this definite quantity, the curd slowly redissolved.

It was observed that " the amount of acid required to produce the maximum rate of precipitation of the casein is inversely proportional to the dilution of the milk," indicating a stoichiometric relation between the casein and HCl, the latter combining with calcium and so throwing down the casein originally combined with the calcium. The number of c.c. of N/10 HCl necessary for 10 c.c. of diluted milk in order to give the maximum rate of casein precipitation were 2·6, 1·2, 0·85, 0·6 and 0·5 for dilutions of 2, 4, 6, 8 and 10 respectively.

[1] Spolverini, *Lait*, **10,** 21 (1930).
[2] Arthus, *Arch. intern, physiol.*, **18,** 133 (1921); Terry, *Arch. Pediatrics.*, **38,** 465 (1921).
[3] *Biochem. Z.*, **102,** 98 (1920). Cf. Demuth and Edelstein, *Z. Kinderheilk.*, **34,** 65 (1923); Eckhardt, *Arch. Pediatrics.*, **42,** 761 (1925); Rudd, *Brit. J. Exptl. Path.*, **11,** 240 (1930).
[4] Kugelmass, " Clinical Nutrition and Feeding in Infancy and Childhood," (Philadelphia and London, 1930), p. 81.
[5] *J. Biochem.* (*Japan*), **1,** 433 (1922).
[6] *Trans. Faraday Soc.*, **19,** 106 (1923).

A second precipitation was also noted by Anderson, the casein redissolved by further acid being again precipitated at a much higher acid concentration. The greater the dilution of the milk, the more the acid required in order to produce an immediate second precipitation of casein. Using 2·6 N. HCl, the least amount which must be added to 10 c.c. of the milk in order to produce immediate second coagulation, was 0·3, 1·1, 1·9 and 10 c.c. for milk undiluted and diluted 1, 10 and 50 times respectively. Such behaviour is suggestive of mass action and the precipitation of casein chloride.[1]

Of the greatest importance in infant feeding is the character of the casein curd from milk, especially where cows' milk is fed. Buckley [2] found that there was a wide variation in the *toughness* of the curds produced by the milk of different breeds of cows when the milk was treated with HCl or rennin. In this respect the milk of Holstein and Ayrshire cows was better than that from Jersey and Guernsey cows. This was confirmed by Hill [3] who carried out an extensive study under the heading: "The Physical Curd Character of Milk and its Relationship to the Digestibility and Food Value of Milk for Infants."

PHYSICAL CHARACTER OF MILK CURD

Hill's method was to coagulate undiluted milk at 35° C., using a coagulant consisting of a mixture of 3 parts of 0·6 per cent. solution of 1-to-3,000-scale pepsin to 1 part of a solution of $CaCl_2$ containing 378 gr. of dry granular $CaCl_2$ per litre of solution.

A special curd knife served for the mechanical determination of the curd toughness. Duplicate 100 c.c. samples of freshly-drawn milk were brought to 35° C. in special glass jars, the curd knife placed in position, 10 c.c. of coagulant added with careful attention to agitation details, and after 10 minutes a spring balance was hooked through a loop in the knife. The latter was then drawn through the curd by a slow and even tension, the amount of tension required being read directly on the balance.

The first surprising result noted by Hill was the amount of variation which can be obtained in the toughness of the curd from the milk of cows in the same dairy herd and of the same breed. "The curd character of the milk appears to be an individual characteristic of the cow rather than a breed characteristic," and cows from the same breed give milks with curds ranging from very soft, resembling buttermilk, to very hard and rubbery.

There is even a day to day variation in curd character of cows' milk, though this is less marked than the variation between the milks of different cows. In the case of milks from different cows the curd

[1] Cf. Loeb, *J. Gen. Physiol.*, **3,** 548 (1921).
[2] Md. Agr. Exp. Sta., *Bull.*, **184,** (1914).
[3] *J. Dairy Sci.*, **6,** 509 (1923); Utah Agr. Exp. Sta., *Bull.*, **207** (1928).

character may show differences quite clear to the eye and not needing quantitative determination. (See Fig. 34.)

Amongst other interesting results obtained by Hill we may notice that removal of the fat from milk increases the curd tension, although there is no direct correlation between the fat content of milk and the curd tension. Fat in milk tends to soften the curd and it is recognised that occasionally infants that fail to digest whole cows' milk will thrive on " top " milk with its greater cream content. In this connection Bergeim [1] and his co-workers had already reported that skim milk gave larger and tougher curds than whole milk in the human stomach.

Hill's work has been applied in various American clinics with excellent results, and his curd test seems to be definitely established as an index to the comparative digestibility of milk by delicate



FIG. 34.—Difference in texture of curd from hard- and soft-curdled milk.

infants. Contrary to the consensus of opinion, the milk from a mixed herd appears unsuitable for infant feeding when compared with the soft-curded milk of one cow. Although it is difficult to fix an upper limit for the value of the curd tension, it has been consistently observed that milk possessing a curd with tension less than 20 gm. gives excellent feeding results.

TYPES OF CASEIN FLOCS

An interesting investigation into the type of casein floc thrown down by dilute HCl (physiological range) on adding to a calcium : casein solution has been published by Loebenstein.[2] His slightly opalescent, bright yellowish-brown solution was prepared by mixing 3·6 gr. casein in 100 c.c. lime water (CaO = 1·2 — 1·25 per cent.), and stood 24 hours before use.

The following table from Loebenstein's paper has an intimate bearing on the problem of colloidal protection in milk.

[1] *Am. J. Physiol.*, **48,** 411 (1919).
[2] *Kolloid-Z.*, **32,** 264 (1923).

Table 57. Action of HCl on 3 per Cent. Calcium-Casein Solution.[1]

(Temperature 37° C.; Time of Observation = 2 hours).

Test.	HCl (per cent.)	Depth of Floc. (mm.).	Nature of Flocs.	Duration of Flocs. (Mins.).	Degree of Turbidity.	Remarks.
1	0·05	3	Separate thick flocs . . .	About 5	Milky.	
2	0·1	24	Coarse dry coagulum, with rifts .	5—10	Cloudy serum.	
3	0·15	11	Thin flocs, very compact . .	About 10	Similar to 2 but clearer.	
4	0·175	2—3	Thin flocs	About 10	Becoming clearer.	
5	0·2	0	—	Immediately	Clarified.	
6	0·25	0	—	Immediately	Clarified.	
7	0·3	0	—	—	Becoming slightly turbid.	Gradual turbidity for an hour.
8	0·35	6	Quite loose and woolly . . .	About 45—50	Opalescent.	
9	0·4	20	Loose flocs; woolly . .	50—60	More turbid than 7 and 8.	
10	0·45	21—22	Denser but fine flocs . . .	30—40	About as turbid as 3.	
11	0·5	32	Dense, fine white flocs . . .	About 20—25	Turbid—serous.	Part sinks and part floats.
12	0·55	36	Dense, fine white flocs . . .	20	Cloudier than 11.	
13	0·6	41	Quite dense, fine flocs as white sludge	20	Same as 2.	
14	0·7	Whole mass	Gelatinous white pap . . .	About 10	Milky.	
15	0·8	Whole mass	Gelatinous and denser . . .	<10	Milky.	
16	0·9	Whole mass.	Still denser and gelatinous. . .	About 3	Milky.	

[1] The solution becomes 3 per cent. owing to the dilution in the test.

The presence of 4·2 per cent. lactose made little difference to the results until the acid concentration was 0·35 per cent. In this case there was no floc (as compared with a floc persisting for 45—50 minutes) and the solution was clear. From 0·4—0·5 per cent. HCl floc was absent and the slight cloudiness lasted only 20—30 minutes. With 0·55 per cent. the depth of floc was 4—5 mm. (as compared with 36 mm.) and was quite loose (as compared with the denser floc with acid only), and persisted about twice as long. Thereafter, the lactose made no difference to the previous results. Thus, lactose tends to inhibit floc formation over the acid range 0·35—0·55 per cent. HCl.

In the presence of 0·5 per cent. albumin, the results were the same as with HCl only until the acid concentration reached 0·35 per cent. Over the acid range 0·35—0·45 per cent. there was no floc formation but rather slight turbidity or opalescence. At 0·5 per cent. HCl concentration a loose woolly floc resulted, lasting for 30 minutes. and with further acid the results were the same as in the absence of the albumin. Albumin thus acts in much the same way as lactose.

A mixture of salts, corresponding to the salts present in cows' milk, increased the precipitation of casein over the entire acid range, 0·05—0·9 per cent. HCl, the casein forming a coarse, dry, and tough coagulum, filling the reaction vessel. The presence of pepsin had no effect on the course of the HCl precipitation, but rennin slightly increased the floc formation and the casein solution in the corresponding acid regions.

In the case of milk, when a curd forms it always carries down with it a large percentage of fat.[1] Fatty curds have a tendency to adhere and form large masses, which resist the action of the digestive juices. The significance of the colloidal protection of cows' milk is therefore extended.[2]

THE DIGESTION OF MILK

The retention of milk foods by infants parallels the casein content of the milk, the average time of retention of human milk being 4 hours 25 minutes. Cows' milk once diluted with water is eliminated almost as rapidly as human milk, but whole cows' milk is retained in the stomach considerably longer.[3] Incidentally, the better digestion of human milk as compared with cows' milk probably bears some relation to the higher lipase content of the former.[4]

[1] Anderson, *loc. cit.*

[2] Cf. Alexander, *J. Am. Chem. Soc.*, **32,** 680 (1910); *Ind. Eng. Chem.*, **15,** 254 (1923); Leary and Sheib, *J. Biol. Chem.*, **28,** 393 (1917); Popper, *Pflüger's Arch. f. Physiol.*, **92,** 605 (1902).

[3] Demuth, *Z. Kinderheilk.*, **33,** 276 (1922), and Demuth and Edelstein, *ibid.*, **34,** 65 (1923).

[4] Freudenberg, *Monatschr. Kinderheilk.*, **38,** 101 (1928).

A number of investigations have been made on the behaviour of cows' milk in the human stomach, the outstanding contribution being that by Brennemann [1] who carried out about 100 tests on a young adult, in whom the vomiting reflex was readily elicited by digital irritation of the fauces. Since rapid emptying of the stomach was thus permissible at will, the contents could be examined after any desired time interval. The milk or its modification was fed at about blood heat, a pint being ingested within 2 minutes or a quart within 5 minutes on an empty stomach after rising. Brennemann's observations showed that :—

(A) The coagulation of the milk is independent of the volume ingested.

(B) Coagulation occurs within a few minutes, the first curds being small and gradually increasing by coalescence for about 2 hours. They then decrease in size due to the peripheral digestive action of the gastric juice, but they still persist after 5 hours.

(C) Raw milk leads to very large and hard curds, whereas boiling the milk for 5 minutes leads to the curds being small and soft. Pasteurised milk (20 minutes at 68° C.) is very like raw milk in the character of the curds produced, being only a little smaller and softer.

(D) Reduction of the fat content of the milk causes harder and larger curds. Increasing the fat content of the milk acts in the opposite direction, but delays digestion of the milk.

(E) Cold milk gives the same curds as warm milk but coagulation of the former is slower.

(F) A larger curd results if the milk is drunk very slowly.

(G) Finer, flakier and more porous curds result when the milk is diluted with water prior to drinking.

(H) Alkalies such as lime water, milk of magnesia, $NaHCO_3$ or sodium citrate present in the milk prior to drinking greatly retard coagulation and, if sufficient be present, may entirely avoid the formation of curds.

(I) Pre-coagulation of the milk by means of rennin or lactic acid bacteria leaves the milk in a condition which avoids further curdling in the stomach.

(J) Whilst starch decoctions, such as barley water, on addition to the milk, lead to small curds in the stomach, such carbohydrates as dextrin, lactose, sucrose and maltose have no appreciable influence on the curd formation.

These results have been amply confirmed by other workers and photographs published of the curds formed at various stages of milk digestion.[2]

[1] *Arch. Pediatrics.*, **34,** 81 (1917).
[2] Bergeim, Evvard, Rehfuss and Hawk, *Am. J. Physiol.*, **48,** 411 (1919).

THE EFFECT OF HEATING MILK

The physico-chemical behaviour of milk on heating has already been discussed (see p. 241). The fact that boiled milk gives softer curds in the stomach has led to the occasional use of boiled milk in infant feeding, correcting the vitamin C content by means of orange juice. Such milk, however, seems to cause a tendency to constipation.[1] Hill, in the work on curd toughness already mentioned, found that heated milk invariably gave a softer curd than did raw milk, the average curd hardness being reduced 69 per cent. by heating at 92° C. for 5 minutes. With those milks normally producing very soft curds when raw, the result of such heating was to lead to a very thin curd rather resembling thin buttermilk. His work, too, confirmed that pasteurisation of milk (30 minutes at 62° C.) frequently failed to alter the curd hardness.

Courtney [2] studied the differences in the behaviour of raw, pasteurised, evaporated and dried milk at the hydrogen-ion concentration of the stomach, working at pH values, 3·75, 4·75 and 5·35. The usual results were obtained in the case of raw, pasteurised and boiled milks. Evaporated milks, as would be expected, produced bulky, fluid curds and dried milk (roller process) gave a compact, cheesy curd. Although unsweetened evaporated milk is favoured in infant feeding,[3] recent work on tryptic digestion indicates that the physical character of the curd is not the only factor involved in the greater digestibility of heat-treated milk.[4]

MODIFIED MILKS IN ARTIFICIAL FEEDING

In the artificial feeding of infants, the use of modified milks has the primary aim of rendering the curd softer when it is coagulated in the infant's stomach.[5] The infant should metabolise the artificial food as efficiently as mothers' milk, and " this can often be accomplished by departing widely from breast milk standards." [6]

One of the earliest and most favoured methods of modifying cows' milk was to add lime water. Clark [7] has pointed out that as human milk is poorer in total proteins than is cows' milk, less alkali is required to shift the equilibria over to pH 8·5. The former practice of adding lime water to cows' milk was based on the assumption that human milk was less acid than cows' milk, or that the

[1] Cf. Dennet, *J. Am. Med. Assoc.*, **63,** 1991 (1914); Brennemann, *ibid.*, **60,** 575 (1913); **67,** 1413 (1916).

[2] *Can. Med. Assoc. J.*, **17,** 919 (1927); Alexander and Bullowa, *Chem. News*, **101,** 194 (1910).

[3] Rice, *Arch. Pediatrics.*, **44,** 758 (1927); cf. Kugelmass, *loc. cit.*, p. 78.

[4] Wallen-Lawrence and Koch, *Am. J. Diseases Children*, **39,** 18 (1930).

[5] Hill, *loc. cit.*, p. 24.

[6] Paterson and Smith, " Modern Methods of Feeding in Infancy and Childhood " (London, 1929), p. 53.

[7] " Fundamentals of Dairy Science " (New York, 1928), p. 127; *J. Med. Research*, **31,** 431 (1915).

calcium content could be increased. However, it is realised now that such treatment tends to complete precipitation of the phosphate, this being less assimilable by the infant.

Bosworth also opposes the practice of adding lime water to milk for infant feeding on the grounds that the acidity of human milk and cows' milk is practically identical. In collaboration with Bowditch [1] he has studied the chemical changes occurring in milk to which lime water is added. Addition of 50 per cent. of lime water reduces the soluble CaO of the milk to less than half, the soluble phosphorus to less than one-third and the soluble citric acid to about three-quarters of the original value. Thus, dilution of such milk to twice the original volume or more reduces the soluble Ca and phosphorus to amounts below the normal values for human milk.

ACIDIFIED COWS' MILK

The present tendency is rather to feed acidified milk, where indicated, since the buffer action of the milk is decreased, gastric activity is stimulated [2] and the peptic digestion of the milk proteins is increased. The fine, flocculent curds produced mechanically assist the digestion.[3] Typical acidified milks are buttermilk,[4] milk adjusted to pH 3·4 by means of 0·3 per cent. HCl,[5] milk containing lemon juice (21 c.c. juice to 1 quart) and reacting pH 5·54,[6] and milk containing 1/15th by volume of vinegar, such acetic milk giving excellent clinical results.[7] Lactic acid milk is especially indicated as its tolerance is greatest [8] and it has been fed with valuable results to premature children.[9]

Excess buffers present in milk delay (and frequently prevent) the completion of gastric digestion in infants, owing to the reduction of the acidity requisite for the optimum action of the gastric enzymes, especially gastric lipase. It is more valuable to reduce such buffer action by the addition of acid than by dilution with water,[10] but whilst therapeutically useful, this type of feeding is not recommended for normal infants.[11]

The buffering action of cows' milk (see p. 206) is due to complexes of calcium with phosphates and casein. The reaction [12] of the

[1] New York Agr. Exp. Sta., *Tech. Bull.*, **43** (1915).
[2] Babbott, *Am. J. Diseases Children*, **26,** 475 (1923).
[3] Krauss, *J. Med. Soc. N. Jersey*, **25,** 792 (1928); Flood, *Am. J. Diseases Children*, **29,** 757 (1925).
[4] Dohnál, *Monatschr. Kinderheilk.*, **27,** 58 (1923); Chasseray, *Rev. prat. malad. pays chauds.*, **4,** 1241 (1925).
[5] Orla-Jensen and Spur, *Lait.* **4,** 845 (1924).
[6] Hess and Matner, *J. Am. Med. Assoc.*, **82,** 1604 (1924).
[7] Dunham, *Am. J. Diseases Children*, **29,** 200 (1925).
[8] Weeks, *Arch. Pediatrics*, **42,** 722 (1925); Johnson, *Boston Med. Surg. J.*, **193,** 1231 (1925).
[9] Gleich, *Arch. Pediatrics*, **42,** 432 (1925).
[10] Faber, *Am. J. Diseases Children*, **26,** 401 (1923).
[11] Faber, *ibid.*, **31,** 395 (1926).
[12] Cf. Lucas, "The Modern Practice of Pediatrics" (London, 1927), p. 200;

stomach contents of an infant at the height of digestion of a meal of human milk is pH 3·75, and Lucas points out the interesting coincidence " that the amount of lactic acid ordinarily found in buttermilk, or lactic acid milk, is just sufficient to saturate the buffer substances of the milk to such an extent that the additional acid required to bring the hydrogen ion concentration to the normal average of the gastric contents (pH 3·8) is exactly the same as in the case of breast milk."

The particular acid milk recommended by Lucas is cows' milk (500 c.c.) plus 75 per cent. U.S.P. lactic acid (4 c.c.) with addition of 1—2 ozs. of commercial corn syrup, and this milk may be fed undiluted to infants from birth, and he states that " the introduction of acid milks into the therapy of nutritional disturbances is one of the great advances in the feeding of sick infants within recent years," having particular value in athrepsia and diarrhœa.[1]

PROTEIN MILK

In cases of infants suffering with celiac disease or indigestion (diarrhœa) so-called protein milk or albuminous milk is fed with advantage.[2] The usual form of this milk [3] is buttermilk diluted with one volume of water and two volumes of whole milk, with extra casein added. The lactose deficiency is replaced by 5 per cent. of dextrin-maltose. Protein milk is higher in calorific value than cows' milk, and is primarily designed to present a medium unfavourable to the development of fermentation strains of micro-organisms.

True colloidal protection of the casein in cows' milk is usually practised by adding gelatin, a method very favourably commented upon.[4] Hawk [5] added 1 per cent. of gelatin to milk and observed that the time required for digestion is decreased. The combination with HCl is more complete and the casein curds are softer and finer. The milk is indicated particularly for unusual allergic infants, tuberculous infants and those having poor tolerance for cows' and human milk proteins.

Using the ultramicroscope, Alexander and Bullowa [6] observed that the addition of a small amount of 0·1 N. HCl to cows' milk caused clumping of the ultramicroscope particles, this increasing until even

Davidsohn, *Z. Kinderheilk.*, **9,** 470 (1914). In this connection see also Mengert, *ibid.*, **33,** 85 (1922) and Clark, " Fundamentals of Dairy Science " (New York, 1928), p. 128.

[1] Cf. also Kugelmass, " Clinical Nutrition and Feeding in Infancy and Childhood " (Philadelphia and London, 1930), p. 76.

[2] Blenkle, *Arch. Pediatrics*, **42,** 743 (1925) ; Kugelmass, *loc. cit.*, 78 and 117.

[3] Neukirch, *J. praticiens*, **39,** 602 (1925) ; *Bull. soc. hyg. aliment.*, **13,** 527 (1925) ; Holt *et al.*, *Am. J. Diseases Children*, **10,** 172 (1915) ; Lucas, *loc. cit.*, p. 887 ; Bertlich, *Z. Kinderheilk.*, **9,** 338 (1913).

[4] Hess and Chamberlain, *J. Am. Med. Assoc.*, **89,** 1423 (1927) ; Perlman, *Arch. Pediatrics*, **45,** 14 (1928) ; Neff, *J. Am. Pharm. Assoc.*, **19,** 261 (1928). An historical summary of the subject is given by Elterich, Boyd and Neff, *Arch. Pediatrics*, **47,** 286 (1930).

[5] *Proc. Soc. Exptl. Biol. Med.*, **20,** 269 (1923).

[6] *Chem. News*, **101,** 194 (1910).

Brownian movement ceased and visible flocs resulted. The presence of gelatin delayed clumping and, in sufficient amount, entirely prevented it.

WHEY

The liquor remaining after the removal of fat and casein from milk, as in cheese-making, is termed whey and contains lactose, proteins [1] and salts. A typical analysis [2] shows: water, 93·1 per cent.; lactose, 5·0 per cent.; proteins, 1·0 per cent.; ash, 0·5 per cent.; fat, 0·4 per cent.; lactic acid, 0·10—0·75 per cent. Almost 75 per cent. of the mineral salts of milks are recoverable in the whey.[3]

Thus, whey is very close as regards analysis to skim milk,[4] except that the latter contains about 3·83 per cent. total protein. Two main types of whey exist: (*a*) grain-curd-casein whey, and (*b*) rennet-casein whey. They differ in their salts content, the former containing nearly all the salts of skim milk since mineral acid has been employed (see p. 212), whilst the rennet-casein whey is less rich in salts, about 33 per cent. of the skim milk salts being removed with the curd. Particularly is this true of calcium and phosphorus.

Since whey is almost casein-free and is low in fat, it has been recommended for the feeding of infants suffering from indigestion arising from incorrect feeding. The food value of whey is good and in dried powder form it represents an excellent and easily-tolerated food.[5]

SURFACE ACTIVITY AND VITAMIN POTENCY

Recently several investigators have inclined to the theory that substances containing vitamins act, not because of some inherent chemical constituent, but because of their marked surface activity.

Von Hahn [6] first developed this conception, confining himself to the water-soluble vitamins, and assuming that the action of vitamins is to enable the cells of the intestinal tract to resorb the food contained in the chyme. Investigation supported the view that surface activity is the factor common to vitaminic foods and to substances which can function as vitamin substitutes.

The unit of surface activity is termed by von Hahn as the "Graham," and 1 Gh. is the force which depresses the surface tension of a solvent 1 per cent. Thus $\mathrm{Gh} = 100\left(\frac{\Delta - \Delta'}{\Delta}\right)$ where

[1] See Grimmer and Hecht, *Milchwirtschaft. Forsch.*, **4,** 564 (1927).

[2] Bell, Peter and Johnson, *J. Dairy Sci.*, **11,** 162 (1928); Rossi, *Lait*, **10,** 136; 278 (1930).

[3] Berry, *J. Agr. Sci.*, **13,** 214 (1923).

[4] Bell and Peter, *Ind. Eng. Chem.*, **20,** 511 (1928).

[5] Cf. Orr and Crichton, *Scottish J. Agr.*, **6,** 63 (1923).

[6] *Arch. ges. Physiol.* (*Pflüger's*), **208,** 732, 745 (1925); *Kolloid-Z.*, **36,** 271 (1925) **40,** 321 (1926); *Wochenschr. Brau.*, **42,** 354 (1925); Alexander's "Theoretical Colloid Chemistry" (New York, 1928), Vol. 2, 917.

Δ' = surface tension of the solvent and Δ = surface tension of the solution.

Von Hahn has published tables showing the Gh. values of many foods and correlating these with their known vitamin contents. The actual value of such Gh. figures is somewhat vague in view of the author's statement : " In measuring fruits, etc., the juices were clarified by centrifuging, and the aqueous extracts of flours, etc. were concentrated as much as possible, so that a fluid was obtained which just formed drops." It was significant that in nearly all cases ultrafiltration of the aqueous solution only slightly decreased the surface activity, *i.e.*, the active fraction is molecularly dispersed. Particularly striking is the parallelism between vitamin content and surface activity when one considers a food wherein the vitamin content varies with the treatment. Thus, potato : raw, slow dried, and steamed, had surface activities of 24·3, 14·6 and 9·2 respectively.

Von Hahn summarises the vitamin relations of the ultra-filtrates of foods thus :—

Surface Activity.	Vitamin Potency.
0—1·4 Gh.	*Nil.*
1·5—9 Gh.	Slight.
10—17 Gh.	Moderate.
17—26 Gh.	Pronounced.

Representative features from von Hahn's table of 51 substances are given in Table 58.

TABLE 58. VITAMIN POTENCY OF FOODS.

Food.	Average Gh. Value.	Vitamin Potency.	
		B.	C.
Butter	1·4	—	—
Cod (raw)	1·8	+	—
Rice (polished)	0·0	—	—
Egg-yolk (raw)	29·9	+ +	—
White cabbage (raw)	17·6	+	+ + +
Potato (raw)	24·3	—	+ + +
Tomato (raw)	13·9	+ +	+ + +
Orange juice (raw)	15·6	+ +	+ + + +
Lemon juice (raw)	22·6	+ +	+ + + +
Yeast (autolysate)	25·3	+ + + +	—
Maize (whole grain)	14·3	+ + +	—
Maize (germ)	20·0	+ + + +	—
Maize (flour)	0·0	+	—

The question now arises as to the function of the surface-active substances, and von Hahn believes that vitamin action is a membrane phenomenon. Brinkman and Szent-Györgyi found that a collodion ultrafilter held back the colloid pigment of blood, but became permeable to hæmoglobin if a surface-active substance was present. Hæmoglobin itself is surface-active ; for its permeability, the added surface-active agent must be molecularly dispersed. Both conditions, surface-activity and molecular dispersion, are filled in vitamin-rich foods.

Applying this idea to conditions in the intestinal tract, von Hahn points out that chyme contains food products so dispersed as to possess low diffusibility. The surface-active materials also present so influence the colloidal or molecularly dispersed food substances that they gain entrance into the blood stream by passing through the membrane of the intestinal wall. "The avitaminoid condition is characterised by absence of surface activity in the chyme, so that, gradually, material necessary for the nourishment of the organism can no longer pass through in correctly balanced proportions ; according to Tscherke, the absorption of nutriment is so poor with deficiency of vitamin B, that food acts like poison. A condition therefore develops which is like starvation, but not fully identical with it, since in vitamin deficiency, *i.e.*, with lack of surface-active material, individual substances of smaller molecular weight always occur in the blood stream." On the nature of these substances, *i.e.*, on the diet, depends the development of beri-beri, scurvy, etc.

Von Hahn [1] quotes clinical observations to support his theory, and states that he has satisfied himself by experimental data that a vitamin-free diet strongly depresses the surface-activity of chyme. His later work with Junker [2] concerned the pleomorphic organism (fungus), causing thrush in infants. Here the power of the fungus to invade the body tissues is due to its ability to form mycelia. In culture media containing substances which depress surface tension, or which contain vitamin B, the formation of mycelia is promoted.

It is of interest to note that other data seem to bear out the notion of a vitaminoid condition. Shepilevskii [3] found that a 0·015 per cent. solution of sodium oleate retarded the loss in body weight in the avitaminosis of guinea pigs. He,[4] however, denied a parallelism between the antiscorbutic potency and the surface activity of plant juices. According to Grafe and Magistris [5] the vitamin action of various vegetable food substances is a function of their water-soluble phosphatides and is related to the capillary activity of the latter.

[1] Alexander's "Theoretical Colloid Chemistry," Vol. 2, 928.
[2] *Jahrb. Kinderheilk.*, **121,** 85 (1928) ; *Kolloid-Z.*, **40,** 321 (1926).
[3] *Biochem. Z.*, **204,** 371 (1928).
[4] *Ibid.*, **210,** 334 (1929). See reply by von Hahn, *ibid.*, **222,** 259 (1930).
[5] *Ibid.*, **177,** 16 (1926).

The theory outlined by von Hahn would not account for the potency of the fat soluble vitamins, and in this connection mention should be made of Zwaardemaker's work.[1] He irradiated heart with polonium or soft radium rays and was able to separate substances which were dialysable and ultra-filtrable. They showed no surface-activity.

[1] *Verslag. Akad. Wettenschappen*, **36**, 253 (1927).

CHAPTER IX

COLLOID PROBLEMS IN SUGAR TECHNOLOGY

SUGAR

The manufacture of sugar introduces several problems demanding colloid chemical considerations. The raw products, particularly molasses, contain colloidal contaminants, and technical operations aim at their removal. Again, the use of active carbon to remove colouring matter is a large-scale application of the phenomenon of adsorption from solution. The paramount importance of pH control is reflected throughout.

THE PREPARATION OF CANE SUGAR

There are three main types of cane sugar : raw, white unrefined, and white refined.

(1) **Raw cane sugar** is prepared from the juice of the sugar-cane, which contains [1] about 14 per cent. sugar, 12 per cent. fibre, 72 per cent. water, and 2 per cent. impurities (wax, gums,[2] colouring matters, ash). Paine [3] gives the following typical analysis of sugar-cane juice :—

	Per cent.
Water	83·00
Sucrose	15·00
Invert sugar	0·80
Ash	0·45
Proteins	0·05
Nucleins	0·03
Proteoses	0·01
Amides	0·05
Amino acids	0·12
Other organic acids	0·10
Gums and pectins	0·10
Fibre particles	0·12
Fat and wax	0·10
Earthy matter	0·06
Chlorophyll, etc.	0·01

The cane is pressed by rollers yielding juice and a damp, saw-dust

[1] Fairrie, " Sugar " (Liverpool, 1925), p. 12.
[2] *Vide* Ruff and Withrow, *Ind. Eng. Chem.*, **14,** 1131 (1922).
[3] U.S. Dept. Agr., *Dept. Bull.*, No. **1370,** p. 70 (1925).

like residue of *bagasse* or *megasse*. The juice is strained to remove suspended impurities and then "tempered" with milk of lime before being raised to boiling-point and passed to settling tanks. A mud separates out and is filter-pressed to yield the maximum amount of juice. The thin juice, containing about 82 per cent. of water, now partially clarified, is vacuum evaporated to about 40 per cent. water content. Further evaporation of the thick juice reduces the water content to 20 per cent. with about 80 per cent. sugar in solution, at which stage crystallisation sets in and, finally, the vacuum pan is filled with a mass of crystals and mother liquor, termed the first *massecuite*. The mother liquor, of course, concentrates up the impurities. The mass is now centrifuged and the first raw sugar crystals removed. The mother liquor syrup is again evaporated, yielding the second *massecuite*, which is slowly crystallised in open tanks and then subjected to centrifugal separation. The second raw sugar is mixed with the first raw sugar (ratio 1 : 2), whilst the syrup is again boiled to yield a third *massecuite*, centrifugal separation of which yields factory molasses.

(2) **White unrefined cane sugar** is duller than refined sugar and contains colouring matter temporarily bleached. Well-known varieties are White Javas, Mauritius Crystals, and the Louisiana and Natal varieties, Plantation Whites. It is suitable directly for food purposes.

In preparing this grade, the main object is to remove colloidal impurities. As before, some colloidal matter is removed by "tempering" with lime. Much more effective removal follows the addition of a greater quantity of lime, which on treatment with CO_2 forms carbonate, the settling of which removes the greater portion of the colloids. Another method of clarification is sulphitation, *i.e.*, treatment with lime and SO_2, thus forming a precipitate of calcium sulphite.

(3) **Refined sugar** is produced from raw sugar by washing in a centrifuge ("affination" process) and then dissolving for filtration with infusorial earth. The clear filtrate is now decolorised with charcoal and then evaporated in vacuum pans.

THE PREPARATION OF BEET SUGAR

A detailed account of the large-scale preparation of beet sugar has recently been given by Owen,[1] to which reference should be made.

The extraction of sliced sugar beet (cossettes) with hot water 80° C.) removes sugars by diffusion,[2] together with soluble non-

[1] "The Desiccation of Sugar Beet and the Extraction of Sugar." (London, H.M. Stationery Office, 1927). *Vide* also Fairrie, *loc. cit.*, 24—35, 91.

[2] For a detailed account of the theory of diffusion see Wohryzek "Chemie der Zuckerindustrie" (Berlin, 1928), p. 248 *seq.* Rowland, Brit. Pat. 281,813 (1926), describes counter-current extraction apparatus for beets.

sugars. The extraction of dried beet cossettes yields pure and more highly concentrated solutions, as part of the colloid content was coagulated during drying.

Matured sugar beet has an approximate analysis as follows :—[1]

	Per cent.
Sugar	16—18
Water	75—77
Fibrous matter (marc)	5
Salts, gum, colour	2

The raw juice obtained by extraction contains about 12—14 per cent. sugar and 4 per cent. impurities,[2] and represents an extraction about 99 per cent. efficient. The juice is usually subjected to a two-stage carbonation and sometimes sulphitation also. The filtered, clarified juice is then evaporated as for cane sugar, bearing in mind that normal raw beet juice contains less gum content than raw cane sugar and no uncrystallisable sugars. The ash content is greater but the colouring matter less tenacious than in the case of raw cane sugar.[3]

SUGAR COLLOIDS

The sugar manufacturer finds obvious objections to the colloids present in his solutions.[4] During evaporation they enhance frothing and sometimes flocculate out in the syrups. It is well known that the colloids retard crystallisation [5] and are tenaciously adsorbed on the crystal surfaces, giving rise to lack-lustre, grey sugar.[6] Their presence, too, is favourable to mycological growths on the stored sugar. Such colloids are negatively charged and are flocculated by addition of lime. Raw cane juice of pH 5·0 when limed in the cold to pH 7·0 gives excellent clarification.[7]

Simple heating of the juice to just below boiling and intimately mixing with kieselguhr or diatomaceous earth will on filtration yield a clear juice ready for evaporation to syrup. Dale and Hudson [8] found that 1,200 gallons of juice (*i.e.*, from 6 tons of cane) could be so treated using 10 lbs. of filter-aid per ton. Of course, only the heat-coagulated impurities are removed, but no scum arises on the

[1] Fairrie, *loc. cit.*, p. 27.

[2] Detailed analyses of sugar-beet juices are given by Wohryzek, *loc. cit.*, pp. 216—227.

[3] Cf. Fairrie, *loc. cit.*, p. 91.

[4] Cf. Seip, *Sugar News*, **9,** 81 (1928).

[5] Waterman and Gentil, *Chem. Weekblad*, **23,** 345—438 (1926) ; Dědek, *Chem. Listy.*, **21,** 96 and 163 (1927) ; *Kolloid-Z.*, **42,** 163 (1927). *Vide* also Wohryzek, *loc. cit.*, p. 63.

[6] *Vide* Paine and Balch, *Intern. Sugar J.*, **28,** 472 (1926).

[7] Brewster, *Louisiana Planter Reference Book* II., 55 (1924).

[8] U.S. Dept. Agr., *Bull.* No. **921** (1920). *Vide* also Zerban, Louisiana Agr. Expt. Sta., *Bull.* **173,** 26 pp. (1920), who uses 0·5 per cent. Filter-cel and 1 per cent. Norit, filtering after each treatment.

evaporation of such filtered juice, and no darkening of the resulting syrup occurs.

Walton [1] extended the above observations on mechanical clarification and pointed out that the method effectively removes the impurities which the ordinary method of boiling and skimming only partially removes. The amount of impurity removed increases as the quality of the cane decreases. The removal of suspended matter accelerates the evaporation of juice to syrup.

By simply superheating cane juice to 116° C., Bird [2] showed that an easily filtered precipitate was thrown down, which analysed 58·74 per cent. ash (34·03 per cent. SiO_2, 1·06 per cent. MgO, 47 per cent. P_2O_5). On treating the filtered superheater juice with lime, a second precipitation was effected, easily sedimented by a Dorr thickener. Heating raw juices at the isoelectric point causes coagulation of the sugar colloids, as pointed out by Seip [3] who worked at pH 5·0—6·1.

Farnell [4] has stated that the problem of colloid content is very important in Natal, as the juice from Uba canes is rich in colloids and ash. He describes a sulphitation process wherein the juice has SO_2 added to pH 4·0 (1·5 gr. SO_2/litre) and then addition of lime to pH 6·9. After heating, the precipitate is settled, phosphoric acid being added to assist this. From 81—94 per cent. colloid content is in this way removable from Mauritius juices.

Electrolytic demineralisation of sugar solutions has been investigated by Zieminski,[5] using solutions containing 15 per cent. sugar and 1 per cent. of potassium chloride, sulphate or carbonate. Graphite electrodes were employed in a modified Pauli apparatus, and the salt content was rapidly removed. Beet juice similarly treated yielded a lighter juice, and an easily-removed precipitate permitting a very clean filtrate.

The electro-dialysis of beet-sugar juices has been described by Bergé.[6] Using iron electrodes, it was found that in the chamber which becomes acidic, the iron dissolves forming ferrous sulphate ; KOH forms in the other electrode compartment. Sugar juice is contained in the central compartment and some organic matter is here precipitated. On reversing current, $FeSO_4$ and KOH interact in the central compartment producing a flocculent precipitate of

[1] U.S. Dept. Agr., *Bull.* No. **1370,** p. 35 (1925).

[2] *Louisiana Planter,* **72,** 257 (1924) ; confirmed later in *Intern. Sugar J.*, **30,** 196 (1928). Cf. Cochran and McCrosson, U.S. Pat. 1,606,827 (1926) ; McCalip and Walton, U.S. Dept. Agr., *Dept. Bull.* No. **1370** (1925), p. 21 ; Friedrich, *Deut. Zuckerind.*, **54,** 1038 (1929) ; Harloff, *Arch. Suikerind.*, **28,** 802 (1920).

[3] *Intern. Sugar J.*, **30,** 82 (1928).

[4] *Intern. Sugar J.*, **27,** 254 (1925).

[5] *Przemysl Chem.*, **13,** 429 (1929).

[6] *Sucr. Belge,* **47,** 283, 302 (1928). See also Mayer in Liesegang's " Kolloidchemische Technologie " (Berlin, 1927), p. 118 ; de Briev, Brit. Pat. 280,321 (1926) ; Wolf and Langstein, Brit. Pat. 214,578 (1924) ; Electro-Osmose A.-G., French Pat. 634,493 (1927).

iron hydroxide, which effects excellent removal of the colloids in the sugar solution. The current consumption per ton of beets is 1·25 kw. hours, and the iron consumed is proportional to the current passed.

Lindner [1] has shown that a suspension of colloidal alumina clarifies beet juice at 45°—50° C., and Levy [2] proposes the use of basic aluminium carbonate produced by the interaction of cold solutions of aluminium sulphate and ammonium carbonate. The mixture is filter-pressed to a cake containing 50 per cent. water. It is mixed with crude sugar juice and raised to 85°—90° C., when owing to decomposition of the basic carbonate, flocculent aluminium hydroxide carries the impurities, leaving a clear juice of lighter colour. Albuminous substances are thus totally removed. No sugar compounds are formed and there is no loss of sugar.

Wells [3] recommends 0·4 per cent. of basic aluminium silicate, a positively-charged colloid, which, in cane sugar juices heated to boiling, brings about excellent separation of colloidal impurities. No gum is left in the juice, although lime-sulphur treatment failed to remove gum. Treatment with lime now removes insoluble calcium salts. Clarification, colour and filtration are all greatly improved.

Numerous other methods for coagulating sugar colloids have been advised (*e.g.*, the use of chlorine,[4] monobarium silicate),[5] but the principle involved is the use of a positively-charged colloid or ion to flocculate negatively-charged sugar colloids. With some juices, *e.g.*, from the Uba canes, starch and its hydrolytic products give trouble in the factory. Whilst carbonation does not effect removal, filtration with kieselguhr does, but an extensive filter surface is needed. The great viscosity of the juice is reduced and filtration greatly facilitated by the use of a diastatic enzyme, which liquefies 2,400 times its weight of starch in 30 minutes, over the range pH 6·5—8·0 (optimum 7·0—7·5) at 60°—80° C.[6]

INVESTIGATION OF CANE-SUGAR COLLOIDS

Paine [7] has discussed the disadvantages of the presence of colloids in sugar liquors, and emphasises that " influence on viscosity [8] is the immediate avenue through which the effect of hydrophile colloids is made manifest during factory operations," retarding diffusion, boiling, and crystal growth.

Dealing with investigational methods and criteria he has pointed

[1] *Deut. Zuckerind.*, **38,** 1136 (1914).
[2] *Bull. assoc. chim. sucr. dist.*, **41,** 224 (1923). Cf. Singh and Majithia, Brit. Pat. 244,924 (1925); Nicholson and Beal, U.S. Pat. 1,578,463 (1926).
[3] *Louisiana Planter*, **71,** 394 (1923); cf. Iida, Japan Pat. 37,430 (1920).
[4] Ochi, *Trans. Am. Electrochem. Soc.*, **49,** 147 (1926); Ochi and Kotera, Brit. Pat. 249,191 (1924).
[5] Deguide, Brit. Pat. 249,759 (1925).
[6] Hudson, *Rev. agr. Maurice*, **5,** 80 (928).
[7] *Ind. Eng. Chem.*, **16,** 1252 (1924).
[8] Cf. Helderman and Khainovsky, *Arch. Suikerind.*, **29,** 1229 (1921).

TABLE 59.

Sample.	Weight Dry Substance Ultra-filtered.	Surface Tension.		Gold Number before Ultra-filtration.	Dried Colloids in Sample.		Fractionation of Colloids.	
		Original Solution.	Ultra-filtrate.				Water-reversible.	Water-irreversible.
	(grams)	(dynes/cm.)		(mgs.)	(grams.)	(per cent.)	(per cent.)	(per cent.)
1	1,270	56·0	74·0	2,600 to 2,700	1·0202	0·080	94·9	5·1
2	1,560	57·8	74·0	3,120 to 3,380	0·9168	0·059	75·6	24·4
3	2,080	59·6	—	2,931 to 3,015	0·3962	0·019	43·0	57·0
4	5,720	74·7	74·7	—	0·9463	0·017	17·0	83·0

out that ultrafiltration is preferable to dialysis for separation of the colloids. After ultrafiltration, the colloid residuum is evaporated and dried at low temperature. The dried and weighed residue can be fractionated with water, giving two fractions : (*a*) reversible, (*b*) irreversible, corresponding approximately to emulsoid and suspensoid. Dialysis or ultrafiltration causes flocculation of some of the colloids due to removal of sugar and electrolytes, and this group is included in the water-irreversible fraction.

Data are given relating to surface tension, gold number, colloid content, and water-reversible and water-irreversible fractions. The tests were made on ordinary commercial grades of granulated sugar, the samples progressively decreasing in order of lustre of crystals (Table 59) as $4>3>2>1$, and this is the order of decreasing surface tension and increasing percentage of dried colloids and water-reversible fraction. Lustreless sugar is considered as due to adsorbed emulsoid colloid.

The percentage composition of the colloid matter was determined, and it was found that the per cent. of ash and of Fe_2O_3—Al_2O_3 in the suspensoid fraction increases in the order $4>2>1$, and is thus probably an important factor in the lack of lustre of the crystals.

Cataphoresis. The sugar colloids are negatively charged. Solutions of raw cane sugar (26 gr./100 c.c. solution) were examined by the method of ultramicroscopic cataphoresis [1] and beet molasses were similarly investigated, diluted to a solution containing 0·5 per cent. solids. The following preliminary data were secured relating to the velocity of the colloid particles in μ/sec./volt./cm. (Table 60).

TABLE 60.

Material.	Velocity of Particles.
Raw cane sugar, 1	0·32
,, ,, ,, 2	0·44
,, ,, ,, 3	0·49
,, ,, ,, 4	0·64
Beet molasses, 1	3·09
,, ,, 2	2·91
,, ,, 3	2·53
,, ,, 5	2·88

Surface Tension. The surface tensions of pure sucrose solutions at 25° C. have been determined by Clark and Mann [2] with the following results :—

[1] In this connection cf. Horne, *Ind. Eng. Chem.*, **20,** 1147 (1928).
[2] *J. Biol. Chem.*, **52,** 166 (1922).

Sucrose. (per cent.)	Surface Tension. (dynes/cm.)
0·005	72·16
0·01	72·27
0·05	72·20
0·1	72·14
0·5	72·31
1·0	72·48
5·0	72·57
10·0	72·69
15·0	72·90
20·0	73·11
30·0	73·48
35·0	73·58

The presence of colloidal non-sugars diminishes the surface tension of sugar solutions.[1] In order to exhibit the influence of colloid impurity on the surface tension of sucrose solutions, Paine added varying amounts of reversible colloids from beet molasses to sucrose solutions containing 21·1 per cent. solids by weight (Table 61).

TABLE 61. SURFACE TENSION OF SUCROSE SOLUTIONS (21·1 PER CENT. BY WEIGHT) AT 20° C.

Colloid Concentration. (grams/75 c.c.).	Surface Tension. (dynes/cm.).
0·000	75·0
0·045	73·8
0·090	60·6
0·135	59·9
0·180	58·8
0·225	56·4

That the influence of the emulsoid colloids is to lower the surface tension is reflected in the foaming of syrups.[2] Removal of scum results in raising the surface tension of such syrups, since the colloids concentrate at the air-liquid boundary.

Honig [3] has measured the surface tension of solutions of pure sucrose at 20° C. and finds that the surface tension is raised from

[1] Kutzev and Kotlyarenko, *Zhurnal Sakharnoi Prom.*, **2,** 357 (1928).
[2] A full account of such foaming is given by Wohryzek, *loc. cit.*, p. 344 *seq.*
[3] *Chem. Weekblad.*, **23,** 265 (1926).

72·68 dynes/cm. in the case of water to 75·24 dynes/cm. for a 50 per cent. sugar solution. Similarly, Plato,[1] using the capillary-rise method at 18·5° C., found the surface tension to increase from 72·59 dynes/cm. (pure water) to 77·06 dynes/cm. for a 65·8 per cent. sucrose solution. Such data contradict the earlier findings of Grunmach [2] who reported that increasing the concentration of sugar resulted in a considerable lowering of surface tension.

Since small quantities of gums and other colloid matter mean a pronounced increase in the viscosity of fillmasses and syrups, and interfere with boiling and crystallisation, Lindfors [3] investigated the surface tension of sugar factory products in an effort to establish a factory control method. He found that the surface tension of a 25 per cent. solution of molasses containing much gum was 42·1 dynes/cm., this rising after filtration to 47·6 dynes/cm. Again, a freshly-diluted solution containing 12·6 per cent. of commercial granulated sugar showed a diminishing surface tension with time due to the gradual accumulation of impurities at the surface. Thus the following surface tension values were recorded : 70·4, 70·0, 67·6 dynes/cm. corresponding to fresh solution, and 10, 25 and 40 minutes later respectively.

The available data is as yet slight, but surface tension measurements should undoubtedly help in checking such factory operations as are intended to remove colloid impurities.

BEET SUGAR COLLOIDS

Kruyt [4] has emphasised the importance of colloid chemistry in the beet sugar industry, and Bergé [5] has recently discussed the subject in detail.

Sugar is associated in the beet with colloids, these constituting the greater part of the organic matter present.[6] Pressure of the beet produces a colloid-rich juice, diffusion giving a purer juice.[7] The colloids flocculate in this juice at pH 4·5 and pH 10·5. One cannot extract with hot water at these pH values because of sugar inversion on the one hand and very slow diffusion on the other.

The diffusion juice is purified by addition of lime (2 kg. per 100 kg. beets) and injection of CO_2 at a temperature of 75° C. After passing the liquor through a filterpress, the operation is repeated at 90° C., using $\frac{1}{4}$ to $\frac{1}{2}$ kg. lime per 100 kg. beets. The filtered juice is now brilliant and colloid-free.

[1] *Z. Vereins Deut. Zuckerind.*, **77**, 467 (1927). *Vide* also, Sazavsky, *Z. Zuckerind. Csech. Rep.*, **7**, 378 (1926).

[2] *Ann. phys.*, **3**, 660 (1900).

[3] *Ind. Eng. Chem.*, **16**, 813 (1924). Also a later paper, *ibid.*, **17**, 1155 (1925).

[4] *Arch. Suikerind.*, **29**, 1102 (1921).

[5] *Bull. soc. chim. Belg.*, **38**, 31 (1929).

[6] *Vide* Wohryzek, *loc. cit.*, p. 59 and p. 177, concerning the nitrogenous matter in beet juice.

[7] Cf. Hamous, *Listy Cukrovar*, **48**, 52 (1929).

Heat coagulates the colloids and their flocculation is total at pH 10·5—11·5,[1] corresponding to addition of 0·1 gr. CaO per 100 kg. beets.[2] The excess carbonate acts as adsorbent and as a filter-aid, as may be shown by the use of a Sharples centrifuge, which gives two layers, (*a*) almost pure $CaCO_3$, and (*b*) $CaCO_3$ + organic matter of bluish tint.

The filtrate is concentrated by heating, first-stage evaporation giving a 55 per cent. yield of sugar. Those colloids escaping removal (emulsoid type)[3] now appear and caramel forms. After filtration with active charcoal, further concentration is effected. A molasses results which is rich in colloid matter and this strongly retards sugar crystallisation. Thus, Bergé reports tests with three sugar solutions : (1) blank, (2) containing added colloids obtained by dialysis of molasses, (3) containing 0·25 per cent. Norit and then filtered. All were seeded with sucrose and evaporated under identical time-temperature conditions. The yield of sugar was 33·8, 4·8, and 34·2 gr., respectively.

Beet colloids are adsorbed on the sugar crystals, causing discoloration. Possibly the adsorption is influenced by the pH prevailing.[4]

SUGAR-CANE WAX

Wax occurs on the outside rind of the sugar cane, particularly near the nodes,[5] and appears to be a complex mixture of alcohols or esters of high boiling point.[6] It is found in the filter-mud retained when cane juice is filtered from its scum, and Allen [7] reports a filter-mud containing 7·76 per cent. wax. It also appears in the soft scale formed in evaporators. Geerigs [8] gives the following typical analysis of scale formed in the first effect evaporator :—

	Per cent.
Cane wax	40·2
Calcium phosphate	48·7
Silica	5·7
Undetermined (organic)	5·4

It has been frequently observed that filtration of raw sugar solutions for refining varies greatly in rate, and this variation has been traced to the presence of wax which defecation has not entirely

[1] Aten confirms this, *Rec. trav. chim.*, **45**, 792 (1926).

[2] In this connection it is interesting to note that Baissac (*Planter and Sugar Manufr.*, **76**, 348 (1926)) states that the most desirable swelling of the colloids in cane sugar juices is at pH *ca.* 7·0, and that further liming causes greater swelling with consequent slower filtration rates.

[3] Paine *et al.*, *Ind. Eng. Chem.*, **16**, 1255 (1924).

[4] Paine, *loc. cit.*, p. 1257.

[5] Cf. Bunker, U.S. Pat. 1,309,999 (1919).

[6] Wynberg, *Intern. Sugar J.*, **11**, 521 (1909).

[7] *Ibid.*, **26**, 35 (1924).

[8] " Cane Sugar and its Manufacture " (London, 1924), p. 196.

removed, or which has gained access to the system again from the filter-mud during steaming and washing.[1]

Bardorf [2] has pointed out that the objectionable features of cane colloids mainly reside in the high percentage of the cane-wax complex extractable by acetone and alcohol, but especially acetone. He isolated the wax-complex by hot dialysis through collodion of (*a*) juice, (*b*) froth from the surface of char-filtered liquors (85—88 per cent. purity), and (*c*) the foam from affination syrups. The wax-complex in raw sugars was of the order 0·13—0·21 per cent. The wax is a dull yellow, hard compound, M.Pt 82° C., insoluble in water, cold alcohol or cold ether. It is readily soluble in boiling alcohol. The acetone-soluble fraction melts at 52° C. Bardorf presents data showing that the alcohol and acetone-soluble fractions are readily retained by filter-cel, cloth tissue, and flocculent precipitates.

QUANTITATIVE DETERMINATION OF SUGAR COLLOIDS

Although Rohland and Meysahn [3] employed aniline blue for the determination of colloids in waste-water containing sugar, the elaboration of a method for the quantitative determination of colloids in sugar liquors by means of basic dyes must be attributed to Paine and his co-workers,[4] who investigated the use of Night blue, Victoria blue B, Methyl violet B, and Bismarck brown.[5]

0·5 gr. Night blue is dissolved in 500 c.c. of freshly-distilled water. A preliminary determination is made of the approximate range of flocculation, including the isoelectric point, by mixing the sugar liquor (containing about 100 mg. solids, diluted to 25 c.c.) to a portion of the dye solution (also diluted to 25 c.c.). Examination of the mixed liquors by illumination may show flocs, and the approximate neutral point is indicated when sufficient dye has been added to yield large flocs, which rapidly sedimentate, leaving a light-coloured supernatant liquor.

The exact quantitative examination is now conducted, working with dye concentrations of small differences over a range to include the isoelectric point. The exact value is determined by cataphoresis using an instrument based on that of Mattson.[6] "The final data of the dye test may be conveniently expressed as an isoelectric ratio, *i.e.*, the ratio of the weight (in mgms.) of dye to the weight (in mgms.) of solids, the colloid content of which is electrically neutralised by the dye. The value of this ratio is a measure of the colloid content of the sample within limits."

[1] Geerligs, *loc. cit.*, p. 274 ; Peck, *Louisiana Planter*, **72**, 94 (1924).
[2] *Ind. Eng. Chem.*, **20**, 258 (1928) ; **21**, 366 (1929).
[3] *Z. Vereins. Deut. Zuckerind*, **63**, 167 (1913).
[4] *Intern. Sugar J.*, **28**, 23, 97, 137 (1926).
[5] *Vide* Wilkinson and Hoff, *J. Phys. Chem.*, **29**, 808 (1925).
[6] *Kolloidchem. Beihefte*, **14**, 309 (1922).

The following data illustrate the method, examining the colloids in beet molasses. The solids = 193·8 mgm. and the total volume is 100 c.c.

Dye in Mgms.	Order of Magnitude of Flocculation.	Migration Velocity of Colloid Particles.	pH at Isoelectric Point.
5·0	+	−0·41 μ/sec/volt/cm.	
5·5	+ +	−0·35	
6·0	+ + + +	−0·22	
6·5	+ + + +	0·00	6·23
7·0	+ + +	+0·26	
7·5	+ + +	+0·39	

$$\text{Isoelectric ratio} = \frac{6{\cdot}5}{193{\cdot}8} = 0{\cdot}0335.$$

Similarly, using 500 mgm. of raw cane sugar, two samples gave isoelectric ratios of 0·0042 and 0·0027, with pH values at the isoelectric point as 6·21 and 6·29 respectively.

Paine's experiments showed that for dye-flocculation tests to be strictly comparable, all solutions should be adjusted to the same pH value (a value close to pH 6·0 being preferred), by adding either acid or alkali to the sample before proceeding with the dye test. The following table illustrates the need for pH adjustment; the total volume was 100 c.c. and the solution contained 500 mgm. raw cane sugar. In each instance there were 2·1 mgm. of dye used.

Migration Velocity of Colloid Particles.	pH.	Remarks.
+ 0·59 μ/sec/volt/cm.	4·78	Flocs do not settle.
+ 0·48	4·98	Good flocculation.
+ 0·37	5·31	,, ,,
+ 0·22	5·73	,, ,,
0·00	6·10	Isoelectric point.
− 0·14	6·35	Good flocculation.
− 0·72	6·77	,, ,,
− 1·37	7·22	Flocs do not settle.
− 1·57	9·54	No flocculation.
− 1·57	9·55	,, ,,

The limits for flocculation are thus pH 4·78 and pH 7·22.

Sucrose concentration has little influence on the flocculation of the colloids in the dye test, as shown when the total solids content in a volume of 100 c.c. was increased from 500 —13,000 mgm. by adding colloid-free sucrose. Paine points out that " while the dye test gives only comparative values it is possible by ultrafiltration to establish factors by which they can be converted into direct colloid values."

THE TECHNICAL REMOVAL OF SUGAR COLLOIDS

In the manufacture of raw cane sugar, defecation or neutral-sulphitation is practised, whilst in making white refined sugar, acid-sulphitation and carbonation are both employed. Beet juice is usually subjected to a two-stage carbonation with optional sulphitation.

Defecation. This is the "tempering" with lime of sugar juice followed by heating.[1] Addition of milk of lime to boiling cane juice yields a precipitate,[2] such precipitation being an optimum with correct lime control. The precipitate readily settles out leaving a clear supernatant liquor.

The precipitate which forms is calcium phosphate, and addition of phosphoric acid is necessary with some juices insufficient in this respect.[3] The precipitate sweeps down colloidal impurities and both by adsorption and mechanical action removes the colloid matter, as proved by examination under the microscope.[4]

The question of the desirable pH value for maximum defecation has been vigorously disputed. Paine and Balch [5] state that maximum clarification and minimum inversion losses occur when the juice is limed (hot or cold) at pH 8—9, the exact value varying with different juices. They found that when the juices were limed to pH 8·4 the resultant precipitate contained about 20 per cent. mineral constituents (Ca, Fe, Al, and Mg. phosphates).[6] As sucrose peptises the oxides of iron and aluminium,[7] the initial phosphoric acid content must be sufficient to permit their precipitation. Roxas [8] agrees that the end reaction in clarification has a variable pH depending on the nature of the cane, but mentions that liming cold and then heating to boiling-point gives two points of maximum clarity, viz., pH 5·22—6·50 and pH 11—12. Other workers [9] obtain optimum defecation when the pH of the treated juice is 7·2—7·6.

Britton [10] has dealt with the problem of pH limits in defecation. In a study of the reaction between calcium hydroxide and aqueous phosphoric acid solutions, he found sucrose to function as a buffer

[1] The advantages of hot liming in giving larger flocs, better sedimentation and lighter juices are treated by Seip, *Intern. Sugar J.*, **30,** 82 (1928).

[2] Superheating to 110° C. yields a mud which settles quickly and is more compact than usual; the juice, however, is more turbid and is darker in colour. See Paine *et al.*, *Ind. Eng. Chem.*, **20,** 266 (1928) and Muller, *Bull. assoc. chim. sucr. dist.*, **38,** 239 (1921).

[3] Bomonti, *Hawaiian Planters Record*, **26,** 122 (1922); Farnell, *J. Soc. Chem. Ind.*, **45,** 343 T (1925); Horne, *Ind. Eng. Chem.*, **16,** 733 (1924).

[4] Bond, *Ind. Eng. Chem.*, **17,** 494 (1925).

[5] *Planter Sugar Mfr.*, **79,** 127, 148 (1927).

[6] Bond. *Intern. Sugar J.*, **27,** 311 (1925).

[7] *Z. anorg. allgem. Chem.*, **174,** 61 (1928); *Kolloid-Z.*, **37,** 101 (1925); *J. Ind. Chem. Soc.*, **4,** 117, 131 (1927).

[8] *Intern. Sugar J.*, **26,** 486 (1924).

[9] Honig and Khainovsky, *Arch. Suikerind.*, **35,** 639 (1927).

[10] "Hydrogen Ions" (London, 1929), p. 397 *seq.*; *Ind. Chem.*, **3,** 508 (1927); *J. Chem. Soc.*, **130,** 614 (1927).

against decrease in pH value. Working at 20° C., precipitation of calcium phosphate is delayed until pH 6·7 is attained and 1·5 equivalents of calcium hydroxide are added.

Farnell [1] has investigated the precipitation of calcium phosphate under various conditions. It is pointed out that the P_2O_5 content of cane juice varies from 0·006—0·087 per cent., and that there is a close connection between phosphate content and the efficiency of clarification. If the P_2O_5 is insufficient, the lime will not precipitate enough calcium phosphate to adsorb the sugar colloids. "The critical value for the phosphate content of Hawaiian juice appears to be about 0·03 gr. P_2O_5 per 100 c.c.[2] Above this quantity the juice clarifies easily, whilst below the juice will be dim, the massecuites may boil slowly, and the raw sugar produced may filter slowly in the refinery." The critical amount of P_2O_5 in cane juice for clarification is probably affected by the amount of colloid matter present. For a precipitate to be formed in sufficient amount to effect adsorption of impurities, ordinary juices should contain 0·005—0·01 gr. P_2O_5 per 100 c.c.

Farnell draws attention to the fact that on adding lime to cane juices, the P_2O_5 is more completely precipitated than the CaO. Since the juice contains phosphates and Fe, Al and Ca ions, addition of lime will precipitate $AlPO_4$ and $FePO_4$ before $CaHPO_4$ and $Ca_3P_2O_8$.[3] "If the clarified juice leaves the settling tanks at pH7 or over, practically all the phosphate will have been precipitated." Lime precipitation removes about 11 per cent. of the non-sugars in the juice, which lightens in colour and becomes transparent and yellow. The limiting pH for defecation is fixed by the pH at which destruction of reducing sugars occurs, viz., pH 8·25.

An important paper by Paine, Keane and McCalip [4] concerns the influence of phosphate and colloid contents of cane juice on defecation. Different experimental lots of raw juice were limed to three pH values and the following results noted :—

Av. pH.	Av. Colloid Elimination.	Av. CaO Content of Clear Juice.
7·18	18·3 per cent.	0·2899 gr. per 100° Brix.
7·71	22·3 ,,	0·3163 ,, ,, ,,
8·05	25·3 ,,	0·3572 ,, ,, ,,

The data showed that "the calcium oxide content of defecated

[1] *J. Soc. Chem. Ind.*, **45,** 343 T (1926).
[2] Cf McAllep and Bomonti, *Hawaiian Planters Rec.*, **26,** 122 (1922).
[3] Cf. Patten, *J. Assoc. Official Agr. Chem.*, **6,** 418 (1923); Stanek and Vondrak, *Z. Zuckerind. Czech,* **51,** 1 (1926); Nakhmanovich, *Nauch. Zapiski. Sak. Prom.*, **17,** 265 (1929).
[4] *Ind. Eng. Chem.*, **20,** 262 (1928); cf. King, *The Planter*, **79,** 221 (1927).

juice bears an approximately linear relation to the content of colloids of the reversible type." Colloid elimination is greater, the higher the pH value, and the volume and weight of mud are increased. However, the higher content of lime salts in the clarified juice is distinctly detrimental.

Ultrafiltration of many samples was effected and the fractionation of the colloids into reversible and irreversible type determined.[1] The CaO content, dye value, and the ultrafiltered colloids in the defecated juices are given by the authors in the following table :—

TABLE 62. ULTRA-FILTRATION OF DEFECATED JUICE—COLLOID CONTENT.

Lot.	pH.	Dye Value.	CaO (per 100° Bx.).	Colloids.		
				Irreversible.	Reversible.	Total.
			Gram.	Gram per 100° Brix.		
1	7·48	960	0·2437	0·030	0·414	0·444
	8·15	907	0·2837	0·037	0·400	0·437
2	6·76	1,024	0·2219	0·034	0·307	0·341
	7·44	1,027	0·2558	0·031	0·333	0·364
	7·96	909	0·2878	0·022	0·347	0·369
3	7·00	650	0·2463	0·012	0·244	0·256
	7·62	671	0·2592	0·008	0·236	0·244
	8·10	638	0·3042	0·020	0·207	0·227
4	7·48	654	0·2354	0·012	0·163	0·175
	8·05	658	0·2803	0·031	0·227	0·259
	8·03	665	0·2742	0·010	0·229	0·239
5	7·19	1,054	0·2952	0·041	0·338	0·379
	7·94	1,129	0·3402	0·065	0·372	0·437
	8·50	1,054	0·4009	0·053	—	—
6	7·16	752	0·3473	0·012	0·263	0·275
	7·40	714	0·3478	0·015	0·278	0·293
	7·50	724	0·3463	0·016	0·296	0·312

On the whole, reversible colloid (gumming nature) is increased by higher alkalinity, possibly due to peptisation of excess lime or cane juice pectinous material or by redispersal by lime of colloid

[1] *Vide* Paine, *Ref. Book of Sugar Ind. of World*, **5**, (v), 53 (1927).

matter already flocculated. The presence of colloidal lime compounds is suggested for a future investigation.

In studying the effect of the phosphate content on colloid elimination, the authors found an approximate ratio between percentage colloid elimination and the P_2O_5 content of the juice, which " raises a strong presumption that the elimination of colloids from cane juice by lime defecation is accomplished *primarily* through adsorption of colloids by the precipitated calcium phosphate."

Bond [1] has carried out very interesting experiments on defecation and shown that this process is primarily a function of the phosphate content of the juice in so far as mineral matter is concerned; organic matter in the precipitate is of secondary importance. He first demonstrated the removal of non-dialysing matter by filtration, finding that juices of about 12·7° Brix filtered through paper, filter-cel and glass-wool respectively, gave non-dialysing matter in gr./litre of 0·140, 0·163, and 2·724 respectively. Defecation removed very little gum.

It was shown that the actual amount of precipitate formed is important, too small an amount giving incomplete defecation, whilst, too much means excess duty on the filter-press. The volume of sediment increases as the ratio $CaO : P_2O_5$ increases. The author concludes that " the actions taking place in defecation are influenced by so many varying factors that their formulation will be difficult, indeed, if not precluded."

Sulphitation. There are two processes :—

(A) *Neutral Sulphitation*, where milk of lime is added, throwing down pectin, anthocyan and gum. SO_2 is now passed in to neutrality, giving a maximum precipitation of calcium sulphite.

(B) *Acid-Sulphitation.* SO_2 is added to juice until this is strongly acid, when lime is added (0·4—0·8 lb. on dry basis to 100 gals. juice) to raise the pH value as desired. The SO_2 used is from 0·6—2·5 gr. per litre. The limed mixture is heated to 100° C., the scum removed, and the mixture allowed to settle, calcium sulphite and phosphate forming the precipitates.

Coates and Taggart [2] point out that it is advisable first to remove suspended matter from the juice as it leaves the mill, sedimentation or filtration being practised. SO_2 gas is added to the juice until 10 c.c. require 3·5—4 c.c. 0·1 N. NaOH for neutralisation, using phenol-phthalein. The milk of lime is screened to a fine suspension of about 15° Baumé and, as a rule, it is best to add as little lime as possible, so that a greenish-yellow juice results, which sedimentates in 10—15 minutes after raising to boiling. Leaving the juice just acid is advantageous in respect to the colour of the final syrup. " Juice properly clarified by the sulphur-lime process is nearly

[1] *Ind. Eng. Chem.*, **17**, 492 (1925).
[2] U.S. Dept. Agr. *Dept. Bull.*, **1370**, 28 (1925).

always slightly turbid, and this appearance serves as a guide to the quantity of lime required for effective clarification."

The juice is now evaporated to about 20°—25° Baumé and the further non-sugar scum removed. The syrup may be settled for 5—6 hours, sedimentation being assisted, if necessary, by addition of 1 in 100,000 of sodium phosphate. The syrup is then concentrated by heat as desired.

Britton [1] points out that the addition of lime should be made as rapidly as possible, and in the cold, since the reaction between slaked lime and H_2SO_3 first produces a soluble salt, $Ca(HSO_3)_2$, over a pH range up to 5·0, *i.e.*, where sugar inversion is possible. Whilst Brewster and Raines [2] state that it is only necessary to lime juice after SO_2 treatment to pH 7 to precipitate all the calcium sulphite, Farnell [3] cannot confirm this, whilst for the hot juice in Hawaiian refineries, McAllep [4] recommends pH 8·2 for optimum sulphite precipitation.

Farnell [5] has investigated the precipitation of $CaSO_3$ under various conditions, and found that while colloids were unfavourable, precipitation was favoured by complete neutralisation of the sulphurous acid, long time and high temperature (up to 100° C.) and high initial concentration of H_2SO_3 and $Ca(OH)_2$. Sucrose does not retard precipitation, but it greatly decreases the crystal size (clusters of crossed needles) of the $CaSO_3.2H_2O$. Colloids altered the character of the sulphite thrown down,[6] apparent spheres forming, their size increasing with rising pH value ; coarser spheres are favoured by high initial concentration of H_2SO_3 and $Ca(OH)_2$ and by holding at elevated temperature. The precipitation of the calcium sulphite was the more complete the higher the juice was sulphured before liming back to a given pH value in the hot. Farnell suggests that the precipitation would be more complete in many cases if the pH value of the hot juice be increased from 6·8 (average practice in Natal and Mauritius) to 7·6—7·8. A pH value of 8·6 on hot juice is considered too alkaline to make white sugar, because of darkening of the juice, but this objection is not serious in making raw 96 per cent. sugar.

Carbonation. One can either add a large excess of milk of lime and then treat with CO_2 to a pH value (8·1) which still inhibits redissolving of the $CaCO_3$ or $Ca(HCO_3)_2$, or carbonation can be carried out in two stages : (*a*) carbonate to pH 11—12 and filter off the non-sugars (pectin, gum, anthocyanin) tending to redissolve at lower pH values, and now (*b*) carbonate to pH 8·1.

[1] " Hydrogen Ions " (London, 1929), p. 401.
[2] *Intern. Sugar J.*, **25,** 88 (1923).
[3] *J. Soc. Chem. Ind.*, **44,** 531 T (1925).
[4] *Intern. Sugar J.*, **27,** 382 (1925).
[5] *J. Soc. Chem. Ind.*, **44,** 530 T (1925).
[6] For instances of the influence of colloids on crystal growth see Steenhuissen, *Pharm. Weekblad.*, **67,** 1269 (1930).

The character of the calcium carbonate precipitate is important,[1] a coarse-crystalline precipitate settling quickly. The addition of sodium carbonate to displace calcium from organic combination has been suggested by van der Heide.[2]

THE COLLOID CHEMISTRY OF MOLASSES

The uncrystallisable syrup remaining after preparation of raw sugar and refined sugar is known as molasses, *e.g.*, factory molasses, refinery final molasses, affination molasses, depending on the immediate origin of the non-crystallisable syrup, in which non-sugars have been concentrated to a noticeable degree.[3]

Helderman and Khainovsky [4] investigated the chemical analysis and viscosity of molasses from the defecation, sulphitation and carbonation of Java sugar cane juice before and after treating with kieselguhr or Norit. A considerable reduction in viscosity was noted in the filtered molasses, which showed a decrease in gum content, though this was not as marked proportionately as the decrease in viscosity. Later,[5] it was shown by ultramicroscopic examination that kieselguhr removed 84—93 per cent. of colloid matter and Norit 3·7—95 per cent. The original carbonation molasses showed only about half as many colloid particles as the defecation molasses.

Gundermann [6] investigated the colloids of beet sugar molasses. Solutions of such molasses containing 10—15 per cent. dry substance were treated with acid, when it was observed that most rapid flocculation of the colloid matter occurred at pH 3·2. Heating to 80° C. increased the sedimentation of the flocs. Since clarification, rate of filtration and the decolorising of solutions, accompanying flocculation are optimal at pH 3·2, Gundermann advises that where SO_2 is used for purification of beet juices and syrups, this pH value should be the working condition.

Our knowledge of the colloid chemistry of molasses has recently been extended by the detailed investigations of von Brodowski.[7] The following is the analysis of the beet sugar molasses used in his research :—

[1] Littlemore, *Chem. Eng. Min. Rev.*, **13,** 441 (1921); de Haan, *Arch. Suikerind.*, **25,** 475 (1917); Harloff, *ibid.*, **28,** 202 (1920). A detailed study accompanied by photomicrographs of precipitates has been reported by Dědek, *Listy Cukrovar,* **48,** 403 (1930).

[2] *Chem. Weekblad.*, **25,** 274 (1928).

[3] Regarding the various kinds of molasses reference should be made to the standard works on sugar technology, *e.g.*, Fairrie, "Sugar" (Liverpool: 1925), pp. 20, 64, 108; and Wohryzek, "Chemie der Zuckerindustrie" (Berlin, 1928), pp. 521—556.

[4] *Arch. Suikerind.*, **29,** 1229 (1921).

[5] Khainovsky, *ibid.*, **29,** 1344 (1921).

[6] *Chem.-Ztg.*, **53,** 305 (1929); *vide* also Parisi, *Ann. chim. applicata*, **15,** 555 (1925).

[7] *Kolloidchem. Beihefte*, **29,** 261 (1929).

	In Molasses.	Calculated on Dry Substance.
	Per cent.	Per cent.
Water	23·88	—
True dry substance	76·12	100·00
Refractometric dry substance	75·39	99·04
Sugar by direct polarization	49·60	65·20
Sugar by inversion polarization	48·60	63·80
Purity	65·20	65·20
Invert Sugar	0·92	1·21
Sulphate ash	9·66	12·68
Carbonate ash	8·69	11·41
Organic matter (non-sugar)	18·83	24·75
Total nitrogen	1·65	2·17
Protein + propeptone + peptone nitrogen (Rümpler)	0·021	0·027
„ „ + peptone (Rümpler)	0·13	0·17
Protein − nitrogen (Barnstein)	0·059	0·078
Protein (Barnstein)	0·37	0·49
Basicity in per cent. CaO (phenolphthalein)	0·074	0·097
Residue insoluble in water	0·196	0·258

The pH values of the molasses at 15°, 23° and 31° Brix were 8·70, 8·78 and 8·88 respectively. Surface tension measurements were made by the du Noüy ring apparatus,[1] and the following data obtained :—

SURFACE TENSION OF MOLASSES.

Concentration. (° Brix.)	Temperature. (° C.)	Surface Tension. (dynes/cm.)
0	19·0	73·0
5	18·0	62·3
10	19·5	60·4
15	19·5	58·2
20	20·0	57·7
25	19·0	55·0
30	18·0	53·2
40	17·0	51·7
50	18·0	50·4
60	19·0	52·0
76	19·0	60·2

The author has presented in graph form the alteration in surface tension (as ordinates) with time (as abscissæ), and there are distinct maxima (at 5 minutes) for the curves corresponding to concentrations of 15° and 20° Brix, whilst the curve for concentration 10° Brix runs parallel to the time axis. The undiluted molasses, 76·1° Brix, shows a constantly rising value for surface tension with time,

[1] *J. Gen. Physiol.*, **1**, 521 (1918—19).

possibly because the high sugar concentration displaces the surface-active material, or because the ring method is in error at such high concentrations.

The pH value prevailing has an influence on the surface tension of molasses. Brodowski diluted molasses to 22·8° Brix with water and adjusted the pH value with 2N.HCl or NaOH. The following data were obtained at 17° C. (see Fig. 35).

Surface Tension of Dilute Molasses.

pH.	Surface Tension.
	dynes/cm.
1·84	53·3
4·45	54·1
5·14	57·5
6·71	60·2
7·60	59·8
8·78	57·9
8·79	57·7
9·14	56·8
9·59	55·7
10·81	51·5
11·69	52·5
13·04	44·3

Surface tension is a maximum at the neutral point (pH = 7) with a second, lower, maximum at pH 11·8, but why this should be,

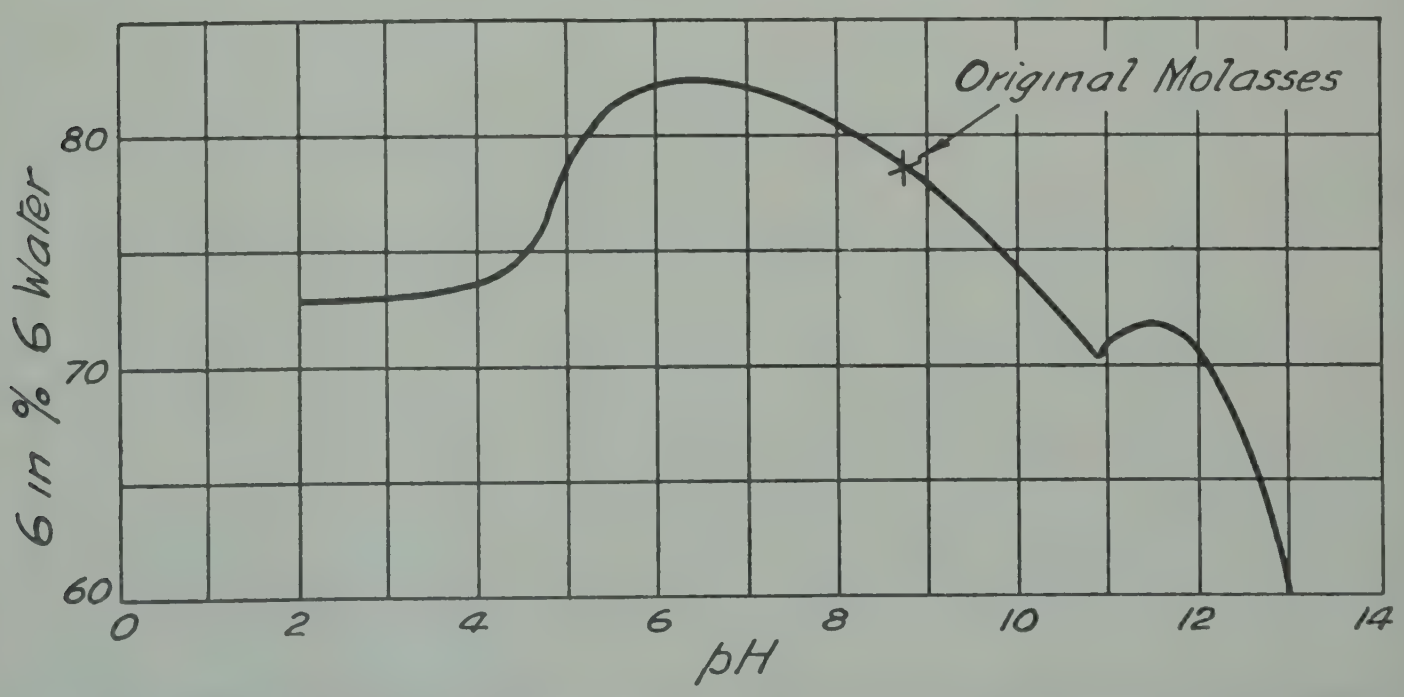


Fig. 35.—Change in the surface tension of a sample of molasses of 22·8 Brix with varying pH. The ordinates show surface tension values as percentages of the surface tension of water.

is not yet clear. The surface tension maximum at pH 7 is explained as being due to there being a minimum concentration of free organic

acids and bases, and these compounds are the most surface-active. Addition of acid to the molasses produces free organic acids, until at pH 4·5 this is complete and the lowering of surface tension remains constant. Similarly, NaOH sets free organic bases, which also lower the surface tension, but to a greater extent than the organic acids.

The viscosity of molasses was found to be a function of the concentration and to be influenced by change in pH value. From pH 1·84 to pH 9·59 viscosity is constant, but thereafter it increases with increase in the pH value. Thus, the relative viscosities of a 22·8° Brix solution at 35° C. were 1·90 and 2·32 at pH values of 1·84 and 13·04 respectively.

THE COLOUR OF MOLASSES

The colour of molasses is due to the colloid matter present, and the isolated colloids gave darker solutions when the medium was alkaline than when it was acid. The effect of the pH value on colour is shown in data reproduced from Brodowski. :—

COLOUR OF MOLASSES AT DIFFERENT pH VALUES.

pH Value.	Colour in ° Stammer.
1·90	302
4·12	312
4·83	351
6·47	393
7·06	389
7·91	391
7·80	406
8·37	409
8·55	421
9·73	437
10·27	448
12·20	448

It was observed that the irreversible colloid A_3 (see p. 313) which yields the darkest colour, is coagulated at about pH 4, and accordingly has no further influence on the colour of the molasses. From pH 4 to beyond pH 5, this colloid wholly dissolves, giving a dark, almost black solution, and the colour curve exhibits a steep rise. The titration curve of colloid A_3 showed a kink between pH 10 and pH 11. Assuming that here is the saturation of the last acid radicle, then further addition of alkali will not effect colour. It is significant that the colour curve changes at pH 10·3.

THE COLLOIDS IN MOLASSES

Brodowski investigated the several colloids present in molasses by the method of hot dialysis [1] for 103 hours, commencing at 40°—50° C. and raising the temperature after 32 hours to 60°—70° C. The residue after dialysis was a dark brown, very cloudy solution, acid to litmus, which brightened on acidifying with dilute HCl, further addition of acid causing turbidity and, finally, a flocculent precipitate.

The dialysed solution when filtered, gave an insoluble residue, which was washed with warm water and dried. The filtrate was evaporated to small volume and then made up to 100 c.c. with distilled water. Quantitative data on the yields from 203 gr. of molasses are given as :—

Water-insoluble Colloid, A, (0·5083 gr.)=0·25 per cent. on molasses

or

=0·33 per cent. on dry substance.

Water-soluble Colloid, B, (2·6460 gr.)=1·30 per cent. on molasses

or

=1·74 per cent. on dry substance.

Colloid A was a grey-brown powder with an ash content of 27·42 per cent., not completely soluble in hydrochloric or nitric acid. The ash was rich in phosphate and contained Fe, Al, Ca, Mg, Na (traces) and SiO_2. On extraction with ether a solution is obtained which on evaporation yields a yellow, greasy substance of low M.Pt. Apparently it is a water-insoluble, ether-soluble acid. The residue from the ether extraction was partly soluble in dilute NaOH, giving a dark brown solution and leaving a brown-grey residue.

Colloid B contained much less ash than colloid A, *viz.*, 6·5 per cent. and was completely soluble in HCl. In addition to phosphate the ash contained traces of Fe, K, Ca, Mg and Na. It was demonstrated that 49·5 per cent. of the total colour of the molasses is due to this colloid.

When dried, colloid B was a dark brown, amorphous glistening and brittle solid, containing nitrogen and having a burnt odour but no taste. The aqueous solution reacts acid to litmus, and reduces Fehling's solution. In alcohol it was apparently insoluble, but evaporation of the alcohol yielded a yellow-white residue, very sticky and having a burnt odour and sweet taste. When dried over calcium chloride it crystallised as small spears, which in aqueous solution did not reduce Fehling's solution. Apparently this fraction is saccharose.

The fraction insoluble in alcohol readily dissolved in water, giving a feebly turbid solution.

[1] Electrodialysis has been used by Kameyama and Kato, *J. Soc. Chem. Ind. Japan*, **31**, 201 B (1928).

A small amount of the water-soluble (reversible) colloid became irreversible after repeated evaporation, an observation already noted by Badollet and Paine.[1]

Colloid B is precipitated by alcohol and by saturated ammonium sulphate solution, lead salts and tannic acid. Saturated zinc sulphate merely gives a turbid solution. Reactions for proteins were negative.

Brodowski next isolated the colloids from molasses and worked out a scheme for their separation into several fractions by part-dialysis, obtaining irreversible colloids A and B, and soluble or reversible colloid C. These in turn were fractionated by ether extraction or by pH adjustment into A_1, A_2, A_3, A_4, A_5, B_2, B_3, B_4, C_1, C_2, C_3, C_4. The fractions A_3, B_3 and C_3 were acidic in character, and the fractions A_4, A_5 and B_4 were amphoteric. The reversible colloid C_4 could not be fractionated, although it was established that it is a mixture of at least two colloids. For further details of these fractions, the original communication should be consulted.

COLLOID CHEMISTRY OF THE STEFFEN PROCESS

The Steffen process applies to the recovery of sugar from beet molasses, diluted with water to about 6 per cent. sugar content, by

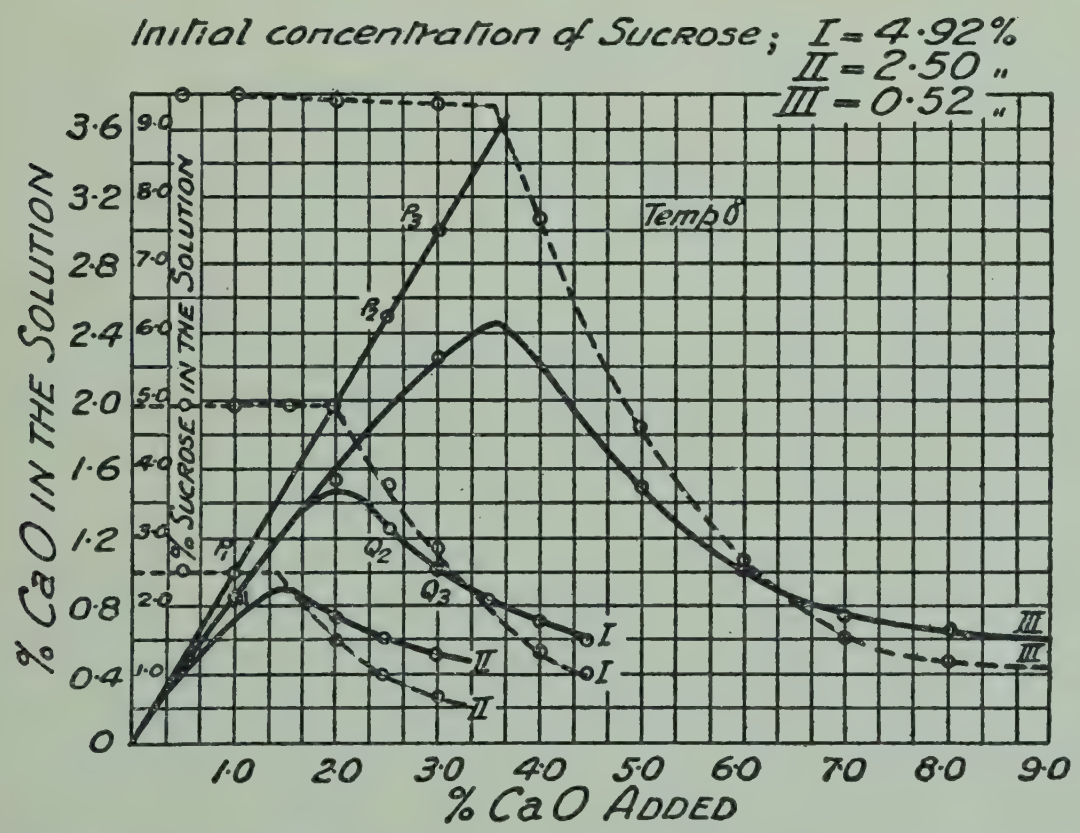


FIG. 36.—Relation between CaO and sucrose solutions. The continuous lines indicate the connection between the quantities of CaO added and the quantity dissolved, the broken lines indicating the connection between the quantity of CaO added and the quantity of sugar dissolved.

adding finely-powdered lime and working below 10° C. The lime and sugar complex, "sucrate of lime," or "trisaccharate," is filtered off and later undergoes reaction in the carbonation tanks whereby $CaCO_3$ is precipitated and the sugar remains in solution.

[1] *Intern. Sugar J.*, **28**, 23 (1926).

Some investigators accept the possibility of a chemical reaction between sucrose and lime to give the compound $C_{12}H_{22}O_{11}$, 3CaO, xH_2O. Dědek,[1] however, by potentiometric measurements of the hydroxyl ion concentration in aqueous solutions of $Ca(OH)_2$ and NaOH, with and without sucrose present, found no ground for believing that the trisaccharate is formed. He suggested that a colloid (adsorption) compound results when finely-divided solid CaO is added to a sucrose solution or when a precipitate occurs by heating a sugar + CaO solution saturated in the cold.

Waterman and van Aken [2] carried out a series of experiments, varying the amount of CaO present in a solution of sucrose of constant concentration, and then estimating the CaO and sucrose in the filtrate. Table 63 is typical of the authors' results, whilst Fig. 36 records graphically their data with respect to 4·94 per cent., 2·50 per cent., and 10·00 per cent. sucrose solutions at 0° C. (curves I., II., and III., respectively).

TABLE 63. TEMPERATURE 0° C. INITIAL CONCENTRATION OF SUGAR 4·94 PER CENT. OF WEIGHT.

Per cent. by Weight of CaO in System.	Per cent. by Weight of CaO in Filtrate.	Per cent. by Weight of Sucrose in Filtrate.
0	0	4·94
0·5	0·41	4·94
1·0	0·86	4·94
1·5	1·23	4·94
2·0	1·50	4·94
2·5	1·25	3·74
3·0	1·00	2·81
3·5	0·90	2·08
4·0	0·71	1·34
4·5	0·61	0·97

The authors consider that their data support the view that colloid-adsorption compounds are formed, their composition being conditioned by the amount of solid CaO present initially. This corresponds with the ideas of Ostwald [3] and Von Buzágh [4] that the quantity of a colloid going into solution varies with the amount of colloid present ("solid-phase" law). In this case, the colloid is CaO, peptised by sucrose. Von Buzágh found that the curve connecting initial amount of colloid with the colloid dissolved shows a maximum, *i.e.*, there is an optimum condition for sol formation.

[1] *Z. Zuckerind. cechoslovak. Rep.* (1925—26), 349.
[2] *J. Soc. Chem. Ind.*, **46,** 411 T (1927).
[3] *Kolloid-Z.*, **41,** 163 (1927); **43,** 227, 249 (1927).
[4] *Ibid.*, **41,** 169 (1927).

Von Buzágh believes that the result is due to the composite character of the colloid, this containing fractions with structural differences, soluble or insoluble in the peptising solution according to its concentration. Ostwald explains the phenomenon by adsorption. For a small amount of the solid phase,[1] adsorption is pronounced and there is a tendency towards precipitation. When there is a larger excess of solid, adsorption is less and little colloid-chemical effect results. Peptisation is a maximum for medium amounts of the solid phase.

COLLOID CHEMISTRY OF CARAMEL

Caramel is the dark brown product [2] obtained by heating sugars. When sucrose is heated to 170°—180° C. until it lose 12 per cent. of its weight, practically pure caramelan is the result.[3] This is a brown gritty powder, melting when dry at 136° C. It reduces silver nitrate and Fehling's solution. Alcohol precipitates it as a syrup from its aqueous solutions. Beal and Zoller [4] prepared caramel by heating sucrose or dextrose at 210° C. for 30 minutes, and effected purification by dialysis in a collodion membrane. The presence of acid assists caramelisation, as does that of various dehydrating agents.[5] Ammonia and its compounds are commonly used in preparing commercial caramel, usually from glucose.[6]

Commercial caramel is a complex mixture of compounds and contains colloidal substances, but the investigation of its colloidal nature has not been studied in detail.

Garino-Canina [7] prepared caramels by heating sucrose to 180° C. until it lost 7·5 per cent. of its weight and found that heating with 2 per cent. $CaCO_3$ gave a product of increased colouring power. The caramel contained negatively-charged colloid material, precipitatable by gelatin but not by tannin. When both tannin and gelatin were present, their flocculent precipitate [8] adsorbed the caramel. For filtration of caramel, the author used cellulose, since this is a negatively-charged colloid. This agrees with Duclaux [9] who observed that nitrocellulose membranes (negatively charged) strongly adsorb caramel in acid solution, but not at all in alkaline solution. Caramel is also adsorbed by aluminium hydroxide and by charcoal.[10]

[1] Cf. Shafor, *Ind. Eng. Chem.*, **19,** 575 (1927).

[2] For a study of the colour analysis by the spectrophotometer see Wayne, *Ind. Eng. Chem.*, **18,** 848 (1926).

[3] Cunningham and Doree, *J. Chem. Soc.*, **111,** 589 (1917).

[4] *J. Am. Pharm. Assoc.*, **3,** 495 (1914); *vide* also Beal and Applegate, *ibid.*, **12,** 495, 850 (1923); Sangiorgi, *Giorn. farm. chim.*, **62,** 256 (1914); Rakuzin and Nesmeianov, *Z. Untersuch. Lebensm.*, **48,** 151 (1924); Wohryzek, "Chemie der Zuckerindustrie" (Berlin, 1928), 78—84.

[5] Cf. Daniel, Brit. Pat., 137,339 (1917).

[6] For full details see Ling and Nanji, *J. Soc. Chem. Ind.*, **41,** 151 T (1922). See also, Yamamura, Japan Pat. 31,668 (1917).

[7] *Notiz. chim. ind.*, **2,** 133 (1927).

[8] Stocks, *Brit. Assocn. Colloid Repts.*, **1,** 76 (1917).

[9] *Rev. gén. colloides*, **4,** 139 (1926).

[10] Rakuzin, *Chem.-Ztg.*, **46,** 770 (1922).

It was shown by Garino-Canina that the lowest concentration of HCl causing flocculation in the caramel in 24 hours corresponds to pH 2·05, but addition of alcohol renders the caramel less stable and raises the minimum pH value for flocculation. He also found that for K_2SO_4 to flocculate caramel, the concentration of K_2SO_4 has to be ten times greater than its usual concentration in wines.

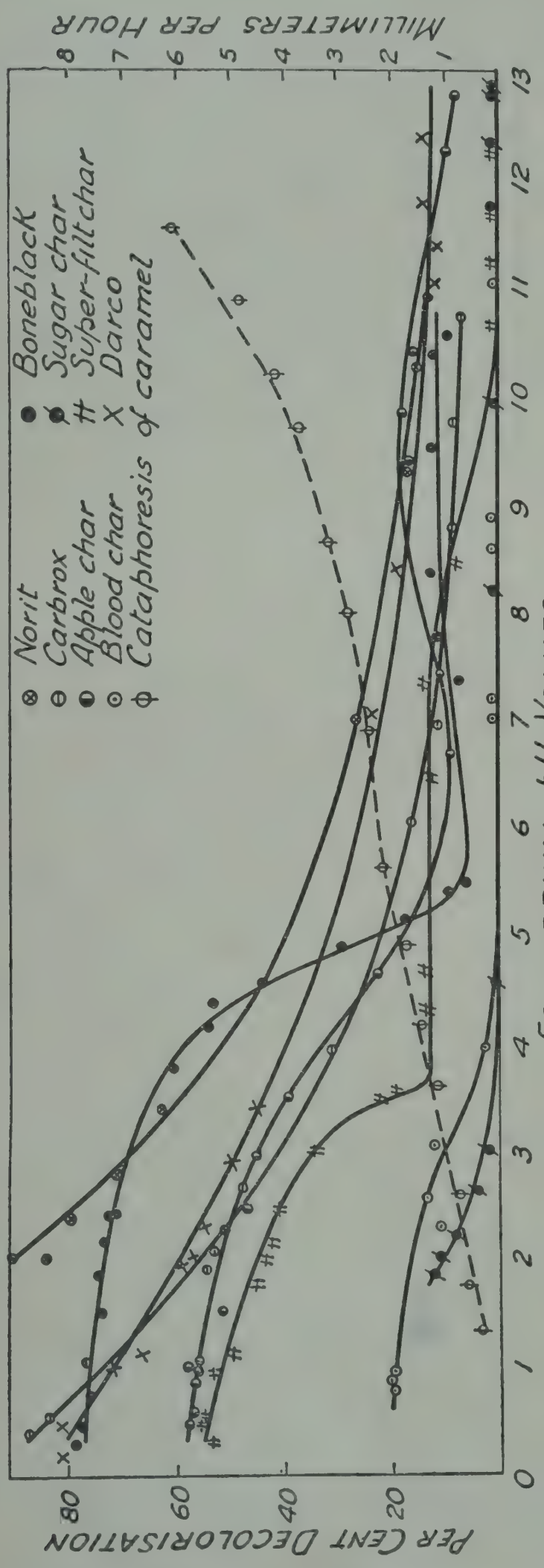


Fig. 37.—Adsorption of caramel by various chars at various hydrogen ion concentrations.

Badollet and Paine[1] found by cataphoresis that caramel prepared from sucrose had a weak negative charge, and when electrical neutralisation was effected by Night blue, the flocculation tendency was very slight indeed. More detailed experiments have been reported by Hague and Willaman,[2] who measured the cataphoresis of a series of solutions of caramel of the same specific conductivity but with different pH values. Inspection of Fig. 37 (reproduced from the authors' paper) shows that the migration velocity decreases continuously as the pH value decreases, and apparently the isoelectric point of caramel is at a low pH value. Although in none of the solutions having a pH value higher than 2·5 was there precipitation even in 18 months, those solutions with pH values less than 2·5 precipitated on long standing, the amount of precipitate increasing with increase in the acidity. No investiga-

[1] *Intern. Sugar J.*, **28,** 100 (1926).
[2] *Ind. Eng. Chem.*, **19,** 944 (1927).

tion appears to have been made regarding the isolation and nature of the colloid material in caramel, or the relation between colloid content and method of caramelising.

There is evidence that caramel retards the velocity of crystallisation of sucrose [1]; whether adsorption of caramel on the sucrose crystals is involved is open to investigation.

THE DECOLORISING OF SUGAR SOLUTIONS

The removal of colour from liquids by means of charcoal or kieselguhr represents the large-scale application of the phenomenon of adsorption. Many interesting problems are encountered in this connection, particularly in the removal of colour from sugar solutions and, to a lesser degree, from oils and fats.

THE COLOUR OF SUGAR SOLUTIONS

Wayne [2] has pointed out that in refining raw sugars we must recognise: (1) grey colouring matter from the cane or due to iron polyphenol compounds formed during the processes of manufacture; (2) red colouring matter due to the action of heat and alkalies on reducing sugars during manufacture; (3) colours from miscellaneous sources.

Zerban [3] showed that certain polyphenol compounds occurring in the sugar cane play an important colour *rôle*, but the colour of raw cane juice is mainly connected with the presence of tannin, iron salts and enzymes. He traced the dark colour of the factory products to several polyphenol derivatives present in cane juice, and found that the presence of ferric salts deepened the colour. The heating of alkaline sugar solutions causes darkening,[4] and incipient caramelisation is also to be reckoned with.[5]

The use of charcoal to remove colour from sugar solutions has long been known, but it is only within recent years that the physico-chemical aspect of the process has been investigated. Numerous forms of decolorising carbons are available for technical use and a considerable literature has grown relating to them. Strict comparison between the results of different workers is rendered difficult owing to the varieties of carbons used, the nature of the colour adsorption test employed, and the failure generally to work under standard conditions. Although the removal of colour is the main thesis, attention is directed to the removal also of colloid material and inorganic constituents.

[1] Kucharenko and Rozovsky, *Planter and Sugar Mfr.*, **75,** 130 (1925).
[2] *Ind. Eng. Chem.*, **18,** 847 (1926).
[3] *Ibid.*, **12,** 744 (1920). See also *ibid.*, **10,** 814 (1918); **11,** 1034 (1919); Harloff, *Chem. Weekblad.*, **16,** 76 (1919).
[4] Herlesóva, *Z. Zuckerind. cechoslovak. Rep.*, **53,** 749 (1929).
[5] Garino and Tosonotti, *Giorn. chim. ind. appl.*, **11,** 8 (1929).

ADSORPTION

Adsorption [1] is a surface concentration and thus is to be distinguished from absorption, which concerns concentration through penetration, *i.e.*, diffusion into the interior. Just what forces are concerned in adsorption still remain open to investigation, but the evidence strongly supports the view that residual valencies at the adsorbing surface are responsible. Stoichiometric relationships are not encountered. Honig [2] has emphasised that adsorption is primarily a function of the dispersion of matter and that the chemical composition of the adsorbing surface is concerned, inasmuch as specific effects are frequent. He found that the heat of wetting runs almost parallel with the adsorption power of carbons of different origin, and concludes that in defining an active carbon, not only should its origin be given, but also its mode of preparation, elementary analysis, specific weight, adsorption value and heat of wetting.

Adsorption is an equilibrium reaction of reversible character and is most pronounced from dilute solutions. Equilibrium is very rapidly reached.[3] Usually adsorption decreases with increase in temperature of the system studied, but the temperature coefficient is quite small.[4]

THE ADSORPTION ISOTHERM

The removal of a solute from solution by an adsorbing surface attains equilibrium or a definite end-point, and for purposes of quantitative data the amount of solute taken up is usually related to the *weight* of the adsorbing material (adsorbent). An empirical general parabolic equation was proposed by Freundlich,[5] which is usually referred to as the adsorption isotherm or concentration function. It is :

$$\frac{x}{m} = KC^{1/n}$$

where x = amount of solute adsorbed.
m = amount of adsorbent.
C = end or equilibrium concentration of solute in the solvent when adsorption is complete.
K and n = constants.

[1] This term was coined by Kayser, *Wied. Ann.*, **14,** 450 (1881).
[2] *Kolloidchem. Beihefte,* **22,** 345 (1926).
[3] Cf. Marc. *Z. Elektrochem.*, **20,** 515 (1914).
[4] The student is referred to the excellent account of adsorption given by Kruyt, " Colloids " (New York, 1930), p. 13 *seq.* See also Rideal, " An Introduction to Surface Chemistry " (Cambridge, 1930), p. 132 ; Bancroft, " Applied Colloid Chemistry " (New York, 1926), p. 108 *seq.* ; Svedberg, " Colloid Chemistry " (New York, 1928), p. 203 *seq.*
[5] For a fuller discussion and numerous references see Freundlich, " Capillary and Colloid Chemistry " (London, 1926), p. 110 *seq.* and 172 *seq.* ; Boutaric, *J. chim. phys.*, **26,** 492 (1929).

The two constants depend on the nature of the adsorbing material and the solute adsorbed. The value 1/n has been found generally to lie between 0·1 and 0·5, and in the case of the adsorption of sugars from their aqueous solution by blood-charcoal the values given by Herzog [1] are 0·539 and 0·474 for fructose and glucose respectively. According to Sanders,[2] values of 1/n as high as 2 have been found by him when dealing with certain sugar refinery syrups.

When the adsorption isotherm is expressed in logarithmic form, the equation becomes one of a straight line, viz. :—

$$\log x - \log m = \log K + 1/n \log C.$$

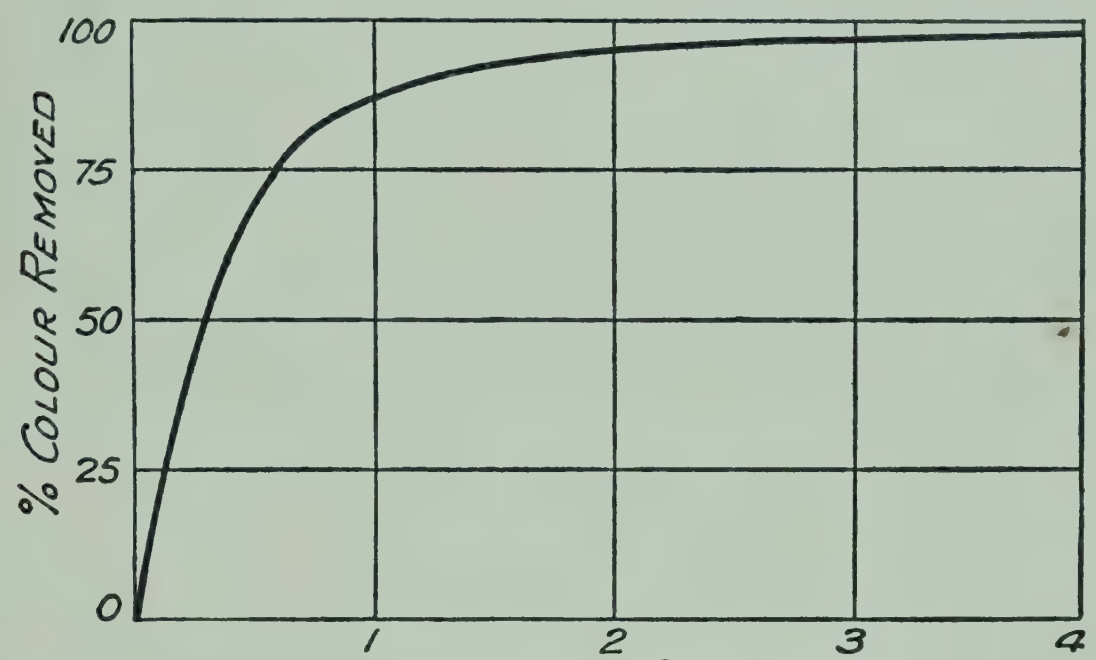


Fig. 38.—The relation between the percentage of colour removed from a molasses solution and the weight of char used.

This equation permits the evaluation of 1/n, since this is the tangent of the angle made by the straight line meeting the abscissa, plotting log C as abscissæ and log $\frac{x}{m}$ as ordinates. Again, the ordinate-intercept is log K.

It should be noted that the adsorption isotherm ignores two possibilities [3] : (1) adsorption of solvent, (2) volume change due to adsorption. Both factors, however, are practically negligible in the circumstances obtaining in technical operations.

To illustrate the adsorption isotherm in connection with sugar decolorisation by carbon, we may instance the data by Sanders.[4] The equation $\frac{x}{m} = KC^{1/n}$ is treated thus :

x = fraction of colour removed by m gr. carbon.
C = ,, ,, remaining in decolorised solution.

[1] *Z. physiol. Chem.*, **60,** 79 (1909) ; cf. Rona and Michaelis, *Biochem. Z.*, **16,** 489 1909).

[2] *Ind. Eng. Chem.*, **20,** 792 (1928).

[3] Williams, *Medd. Vetenskapsakad. Nobelinst*, (2), **27** (1913).

[4] *Ind. Eng. Chem.*, **15,** 784 (1923) ; *Chem. Met. Eng.*, **28,** 541 (1923) ; cf. Sauer, *Intern. Sugar J.*, **26,** 27 (1924).

Fig. 38 reproduces his graph for the removal of colour from molasses, whilst Fig. 39 reproduces the corresponding logarithmic plotting of his data.

Inspection of the adsorption isotherm shows that the extent of

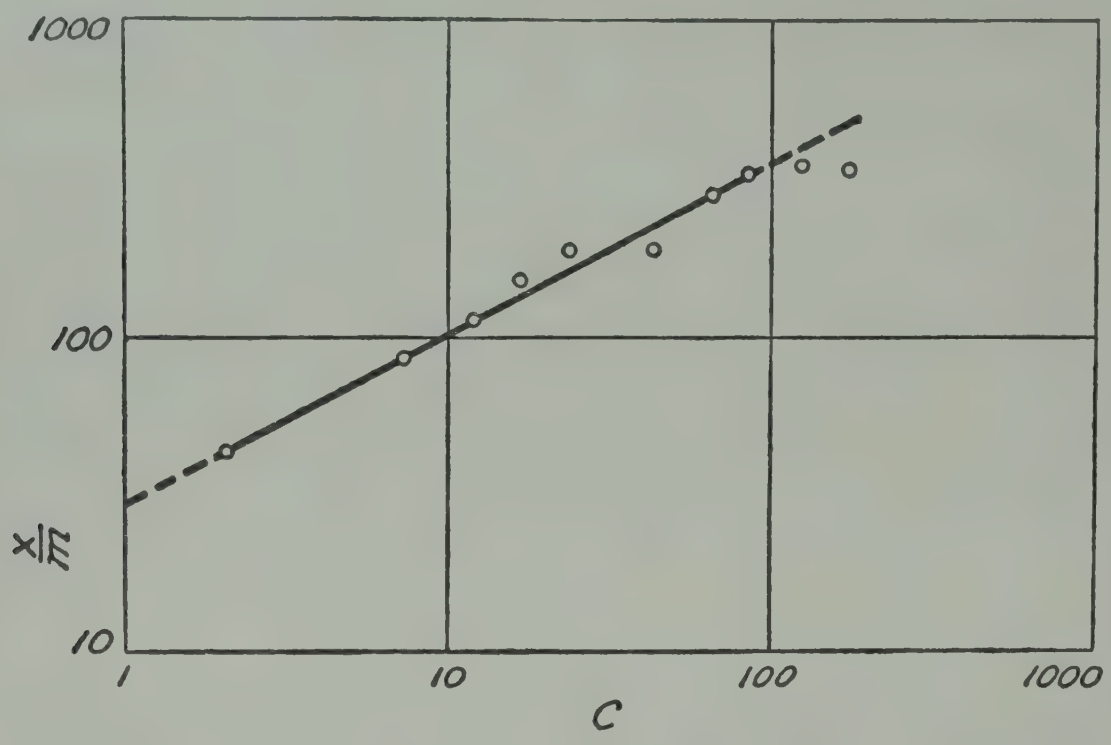


Fig. 39.—The logarithmic plotting of the data used in Fig. 38, but now using colour units instead of percentage of colour.

adsorption is relatively more pronounced in dilute solution, the amount adsorbed increasing with, but more slowly than, the concentration. As Sanders points out, this fact suggests the advantage of counter-current methods of decolorisation, since by such operation,

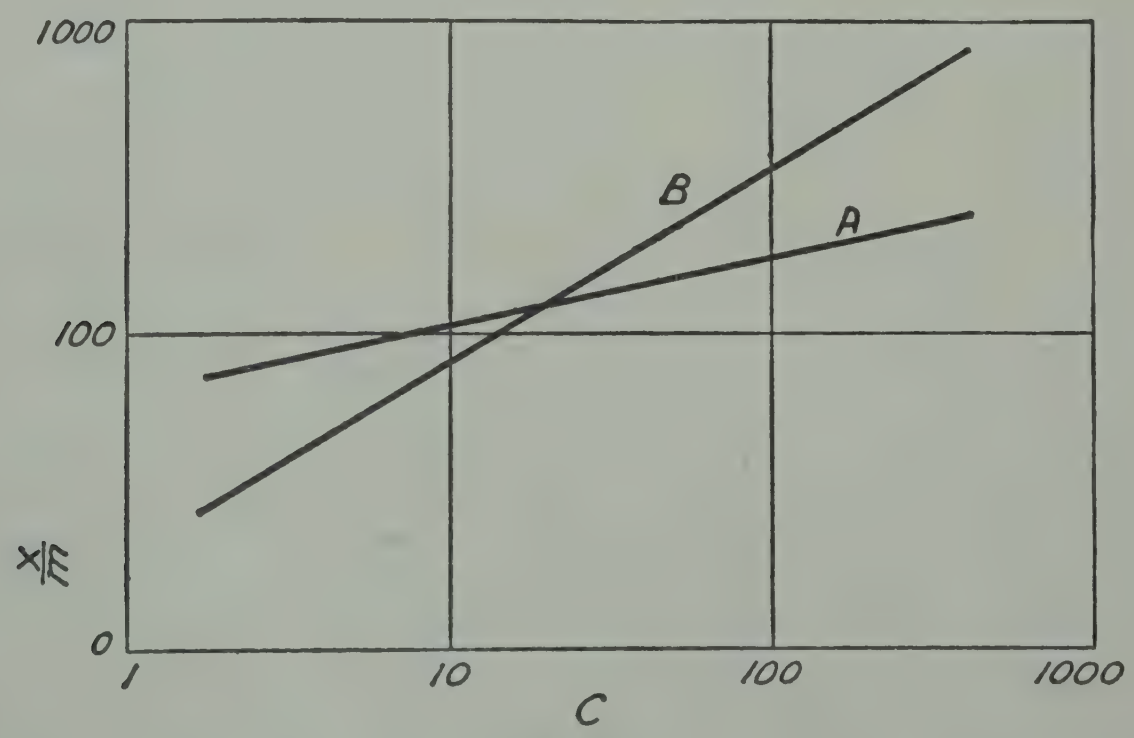


Fig. 40.

fresh carbon meets partially decolorised solution, and so removes more colour than a one-treatment operation of carbon with a deeper coloured liquid.

Sanders refers to Fig. 40 representing the logarithmic plottings for two different carbons. Then " for high degrees of decolorisation,

in a single application, A would be superior to B, weight for weight, at the point of intersection they would be equally efficient, and for low degrees of decolorisation or counter-flow work B would be superior to A." The straight line graphs also show the necessity of testing the adsorption efficiencies of different samples of carbons by choosing different initial concentrations of the colouring matter in solution.

Pointing out that counter-current decolorisation presents technical difficulties, Sanders [1] outlines the stepwise counter-current method or the use of the carbon in two or more stages. Fresh carbon is employed to treat solution already partially decolorised,

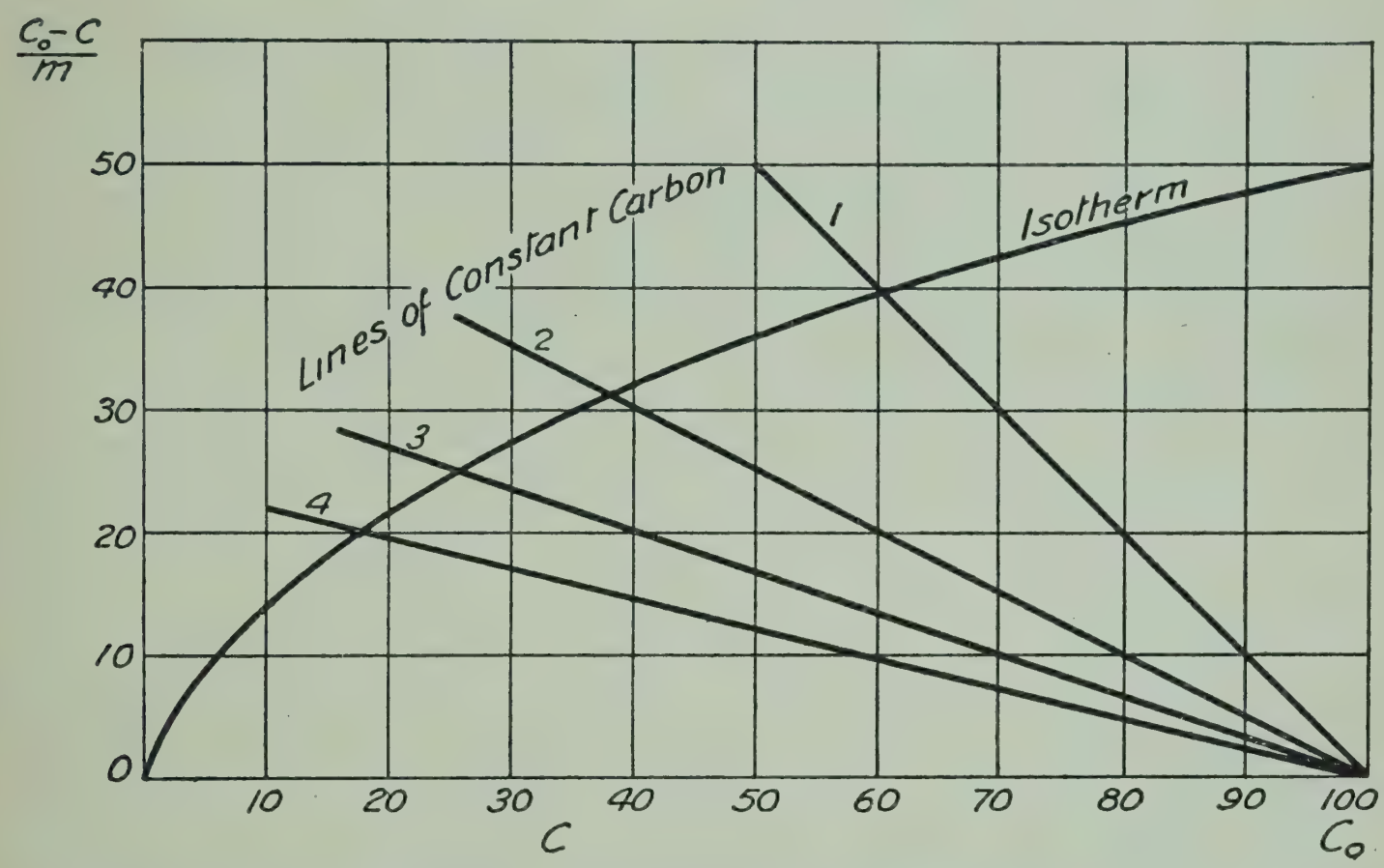


FIG. 41.—Family of isotherms representing the action of different carbons on a given sample liquid, plottings being made on rectangular co-ordinate paper.

and then the carbon (now partly spent) is employed in the first decolorisation of fresh solution.

Sanders proposes a simple modified form of the adsorption isotherm :—

$$\frac{C_0 - C}{m} = KC^{1}/n$$

where C_0 = original colour of the solution,
C = colour of the solution after adsorption process,
$C_0 - C$ = amount of colour removed by m gr. carbon.

He points out that " in any family of isotherms representing the action of different carbons on a given sample liquid all points of constant carbon fall on a common line." Thus, plotting $\frac{C_0 - C}{m}$

[1] *Ind. Eng. Chem.*, **20,** 791 (1928).

against C on rectangular co-ordinate paper, the lines of constant carbon (m constant for a given line) are straight and pass through C_0, whilst on double logarithmic paper they exhibit sharp curvature and become asymptotic to C_0. Figs 41 and 42 are reproduced from Sanders' paper.

Reversing this process, from the isotherm plotted one can estimate the extent of colour removal effected by a given weight of carbon.

Mecklenburg [1] treats the problem of calculating the equilibrium

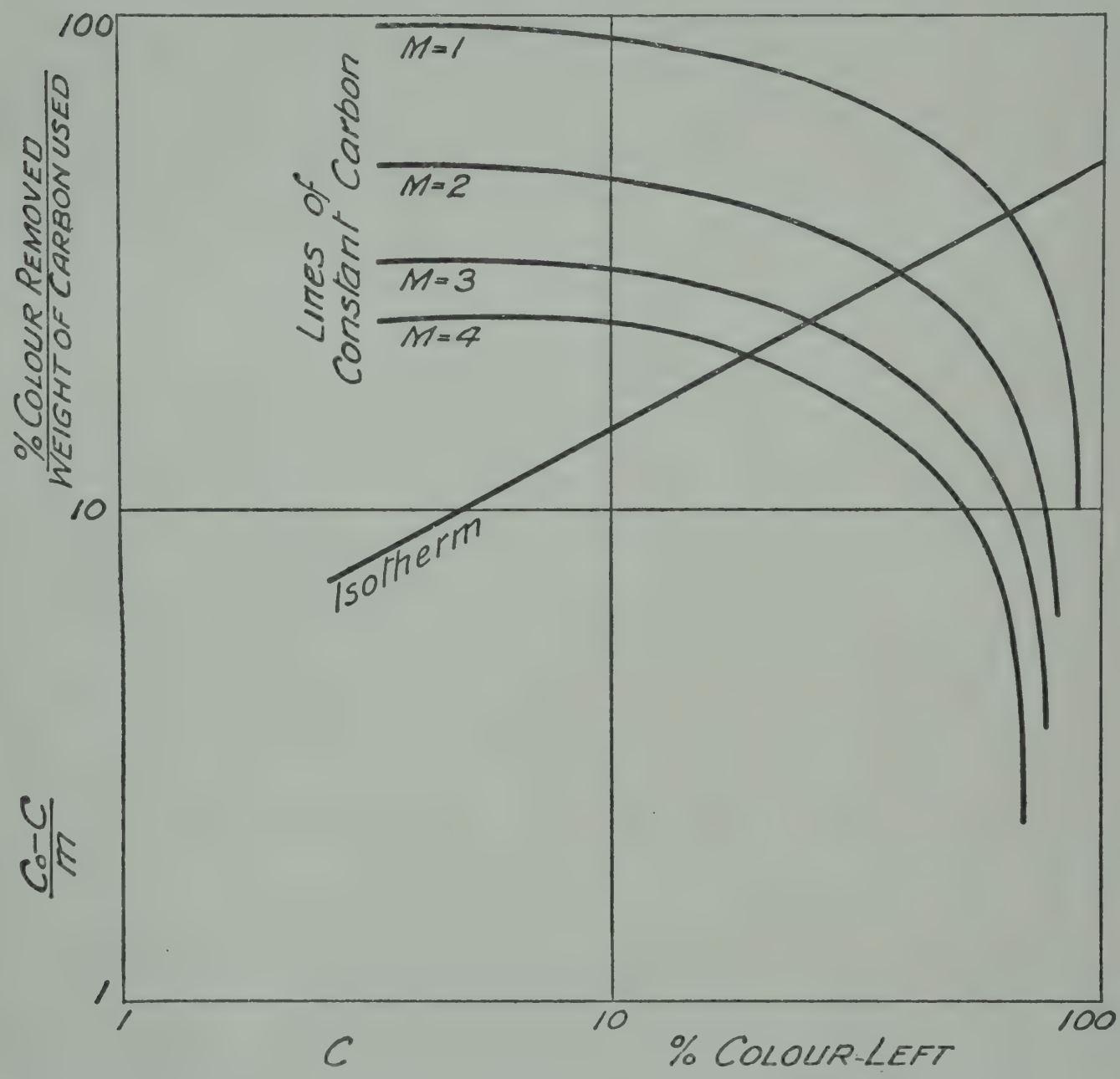


FIG. 42.—Family of isotherms representing the action of different carbons on a given sample liquid, plottings being made on double logarithmic paper.

colour concentration from the initial colour concentration for unit mass of carbon. The adsorption isotherm is obtained by plotting C_0—C as ordinates against C as abscissæ. The value of the original colour of the solution (C_0) is now marked off on the ordinate and the abscissa axis, and the two values joined by a straight line (hypothenuse of an isosceles right-angled triangle). This line cuts the isotherm graph in a point which defines the conditions for adsorption, and the abscissa value of which relates to the amount of colour persisting in the equilibrium concentration following adsorption.

[1] *Naturw. Wochenschr.*, **15,** 409 (1916). See Freundlich, "Capillary and Colloid Chemistry" (London, 1926), p. 178.

Teeple and Mahler[1] used the adsorption isotherm in testing decolorising carbons, working with a Hess-Ives tint photometer, following decolorisation only with the light that is most strongly adsorbed, *viz.*, blue violet. If

$\frac{x}{m}$ = amount of colour adsorbed, x being expressed in colour units,

C = amount of colour in equilibrium (expressed in colour units).

and $\log \frac{x}{m} = \log K + 1/n \log C$,

then this is the equation of a straight line, and K (ordinate-intercept) will be the number of colour units each gram of carbon would hold if the final (equilibrium) concentration of colour in the solution were reduced to unity (1 colour unit). The adsorption of colour by unit mass of carbon is equal to the product of the equilibrium concentration of colour raised to the power 1/n, multiplied by a constant (K) ; the only variable then is the final or equilibrium concentration. The values for the two constants are dependent on the nature of the carbon employed.

Fig. 43 reproduces graphs of data for colour adsorption from a sample of molasses, using ten different carbons. Two special cases are considered :—

(1) What are the comparative efficiencies of the carbons in decolorising the molasses solution to an end value of 10 blue-violet colour units ?

The ordinate-intercepts at the point where C = 10 show, for example, that carbons numbers 1, 2, and 10 hold 198, 191 and 111 colour units per gram respectively. Accordingly, carbon number 10 is worth just 56 per cent. of the price of number 1 as far as decolorising power is concerned.

(2) It is desired to decolorise only to 100 colour units for (say) counter-current work. Which carbon is best ?

From the values of the ordinate-intercepts corresponding to C = 100, the choice of carbon would be number 2, the second best is number 3 and the next best number 1.

The authors emphasise that such comparative values apply *only to the particular solutions studied.* The investigator must determine the efficiency of his carbons for the particular system he is working with.[2]

[1] *Ind. Eng. Chem.*, **16,** 498 (1924).

[2] For example, see Ogilvie, *Intern. Sugar J.*, **30,** 120 (1928) ; Strachan, *Chem. and Ind.*, **47,** 1205 (1928) ; Thomas, *Intern. Sugar J.*, **23,** 162 (1921) ; Turrentine and Tanner, *Ind. Eng. Chem.*, **14,** 19 (1922) ; Honig, *Kolloidchem. Beihefte*, **22,** 398 (1926).

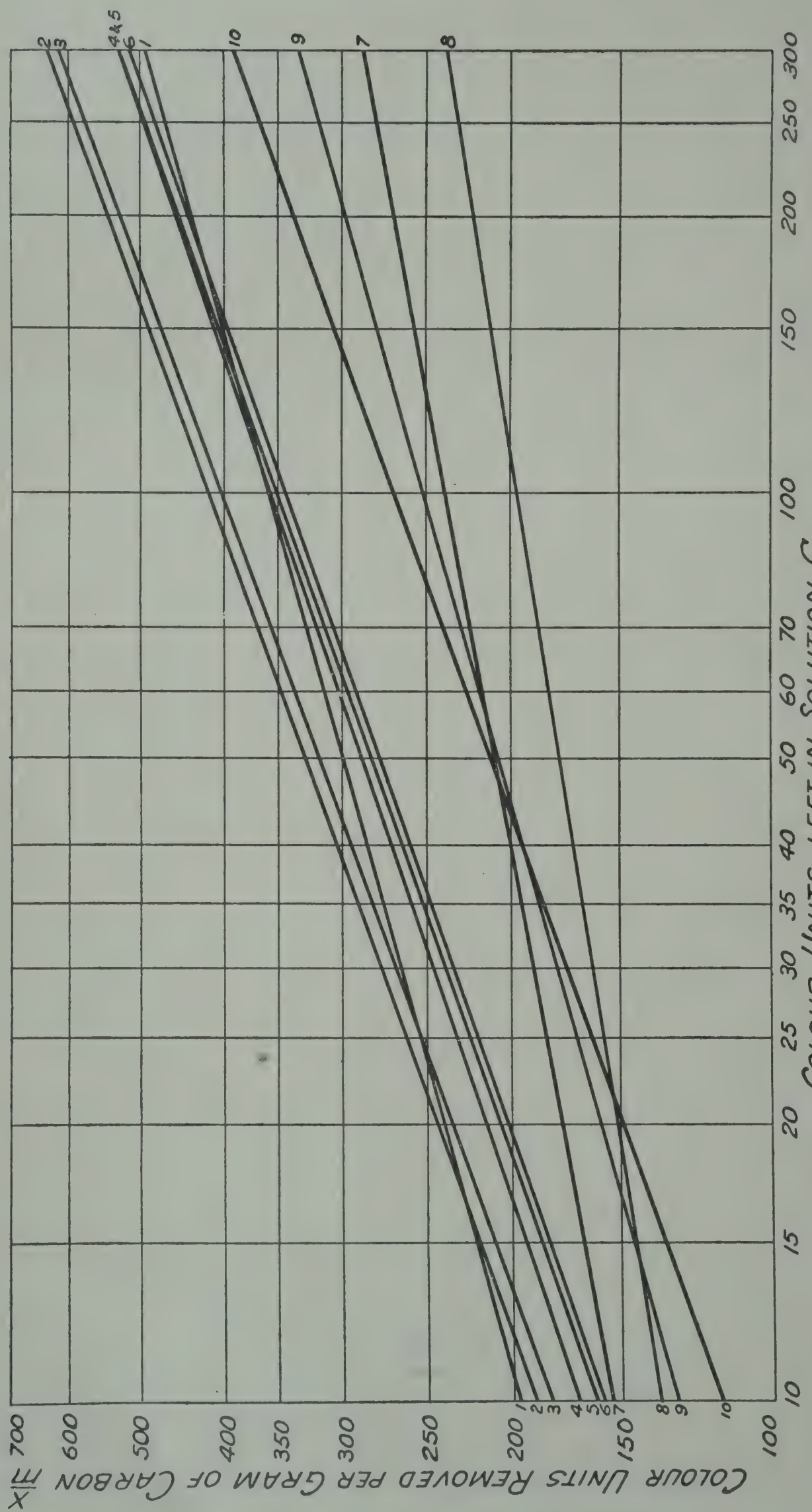


FIG. 43.—Graphs (logarithmic form) of data for adsorption of colour from molasses, using ten different chars.

DECOLORISING CARBONS

Decolorising carbons [1] can be prepared from various sources by appropriate heating. Thus, from : sawdust [2] by prolonged heating at 1200° C. in a covered crucible, lignite [3] by prolonged heating to 1200° C., bagasse [4] heated to 500°—600° C., degreased bones [5] heated to a cherry-red heat for 8—12 hours (" bone-char ").

It is generally agreed that better decolorising chars are obtained from raw material, which are impregnated naturally or intentionally prior to heating.[6] Zerban, Freeland and Sullivant [7] enumerate 39 decolorising chars with details of their manufacture, and point out that the impregnating substances include H_2SO_4, HCl, CaO, $CaCO_3$, chlorine gas, $CaCl_2$, $MgCl_2$, $ZnCl_2$, etc.

Bone black has a calcium phosphate skeleton on which carbon is deposited. Similarly, " Carbrox " prepared by heating rice hulls, has a siliceous content, which is removed by boiling with caustic soda. The resulting washed char is very active.[8] Schneller,[9] investigating the question of impregnation, found that the decolorising power of the final char depended on the quantity of impregnating material, the temperature of heating, and the degree of removal of the impregnation. Zerban and his co-workers [10] made a detailed investigation of impregnating materials and found that both chemical and physical factors are involved, the nature of the impregnating substance being important. The really fundamental factor seems to be the state of aggregation of the impregnating agent at the temperature of charring, and this state should be solid or gaseous, not liquid. It was also found that there must be a vigorous attack on the carbohydrate content of the raw material used and that both this and the impregnating material should be finely divided. It is still not certain whether the impregnating substance functions by preventing the adsorption of non-active hydrocarbons in the char

[1] For historical details see Zerban, Louisiana Agr. Exp. Sta., *Bull.*, **162** (1918) Fairrie, " Sugar " (Liverpool, 1925), p. 158 ; Wohryzek, " Chemie der Zuckerindustrie " (Berlin, 1928), p. 603 *seq.*

[2] Coates, Louisiana Agr. Exp. Sta., *Bull.*, **161,** 8 (1918).

[3] Lotz, *Z. Ver. deut. Zuckerind.*, **62,** 1304 (1911).

[4] Coates, *Ind. Eng. Chem.*, **14,** 295 (1922) ; Clacher, *Intern. Sugar J.*, **16,** 64 (1914) ; Weinrich, U.S. Pat. 455,675 (1891).

[5] Strachan, *Chem. and Ind.*, **47,** 1203 (1928).

[6] That the presence of impurities is essential for a good decolorising carbon is contended by Glassner and Suidu, *Ann.*, **361,** 351 (1908) ; Knecht and Hibbert, *J. Soc. Dyers Colourists*, **32,** 226 (1916) ; Patterson, *J. Soc. Chem. Ind.*, **16,** 608 (1903) ; Tanner, *Ind. Eng. Chem.*, **14,** 441 (1922) ; Hall, *ibid.*, **14,** 18 (1922) ; Horne, *ibid.*, **11,** 372 (1920) ; Bardorf, *Facts about Sugar*, **11,** 351 (1920). See also Naugle, Ger. Pat. 500,582 (1926) ; Metallges A.-G., Ger. Pat. 502,040 (1926) ; von Halban and Schober, U.S. Pat. 1,774,341 (1930).

[7] Louisiana Agr. Exp. Sta., *Bull.*, **167,** 5 (1919).

[8] Zerban, *ibid.*, *Bull.*, **161,** 9 (1918). Cf. Tokunaga, Jap. Pat. 31,604 (1917) ; Shilstone, *Louisiana Planter*, **58,** 364 (1917) and *Intern. Sugar J.*, **21,** 249 (1919) ; Schilov, Dubinin and Toporov, *Kolloid-Z.*, **49,** 120 (1929).

[9] *Louisiana Planter*, **59,** 156 (1918) ; cf. *J. Soc. Chem. Ind.*, **40,** 230 T (1921).

[10] *Loc. cit.*, 14 *seq.*

pores during heating or whether they act as dehydrating agents. Certainly they function so that the final char is desirably porous, *i.e.*, increasing the area of the adsorbing surface.[1] Well-known chars of the above types are radit, carboraffin and carbrox. The important decoloriser known as Norit, is prepared by carbonising organic material and activating then with a gas (CO_2, CO, Cl_2) at 600°—1000° C.[2]

The activation of chars by treatment with gases at high temperatures has received considerable investigation. Chaney [3] adopted the working hypothesis that ordinary amorphous carbon is a mixture of "α-carbon" (active form) and "β-carbon" (inactive form), and that active carbon is formed by chemical or thermal decomposition at 500°—600° C. of carbon-bearing materials, higher temperatures giving inactive carbon. Thus, active char is a form of pure amorphous carbon free from adsorbed stabilised hydrocarbons or β-carbon. In a further communication, Chaney and his co-workers [4] treat very thoroughly the question of activation by gases, and define their test for the "activity" of carbons. This is determined from the amount of iodine adsorbed from 50 c.c. of 0·2 N. iodine—KI solution by 1 gr. of the dried, 200-mesh carbon, after agitating for 3 minutes.[5]

It should be emphasised that the iodine adsorption test, or indeed any arbitrary colour adsorption test, cannot be directly translated into technical practice as an index of the efficiency of carbons to decolorise sugar solutions, etc. A set of samples of carbons needs to be investigated for decolorising efficiency in respect to the actual type of solution eventually to be handled on the technical scale, and the order of efficiency so determined will probably be very different from that as determined by iodine adsorption.[6]

THE NATURE OF "ACTIVE" CARBON

It is realised that volume rather than weight determines the decolorising capacity of chars, and Weinrich [7] finds that equal volumes of different chars exercise the same decolorising effect.

[1] Cf. Lowry, *J. Phys. Chem.*, **34,** 63 (1930); Isobe and Mori, *Bull. Inst. Phys. Chem. Res. Tokyo,* **8,** 801 (1929).

[2] See Wijnberg and Sauer, Brit. Pat. 21,204 (1911); Dědek and Zert, *Z. Zuckerind. cechoslovak. Rep.*, **38,** 17, 25, 33, 41 (1923).

[3] *Trans. Am. Electrochem. Soc.*, **36,** 91 (1919). *Vide,* Ostrejko, Brit. Pat. 106,089 (1918).

[4] *Ind. Eng. Chem.*, **15,** 1244 (1923).

[5] Regarding this iodine test see Davis, *J. Chem. Soc.*, **91,** 1666 (1888); Honig, *Kolloidchem. Beihefte,* **22,** 385 (1926); Firth, *J. Soc. Chem. Ind.*, **42,** 242 T (1923) and *Trans. Faraday Soc.*, **16,** 434 (1921).

[6] Cf. Teeple and Mahler, *Ind. Eng. Chem.*, **16,** 499 (1924); Edelstein, *Z. Zuckerind. cechoslovak. Rep.*, **54,** 257 (1930); Sartorius and Ottemeyer, *Gesundh. Ing.*, **53,** 227 (1930).

[7] *Intern. Sugar J.*, **19,** 406 (1917).

Firth [1] believes that the more active a carbon, the simpler is the carbon molecule.

Numerous workers have advanced widely diverse theories of the mechanism of the activation of carbon. Thus, Shilov [2] believes that the adsorptive power of carbon is due to three different oxides existing on the surface and possessing different adsorptive capacities towards alkalies and acids. In this connection may be noted that Alekseevskii and Musakin [3] find that activation of carbon is greatly increased by treatment, with such oxidising agents as $HClO_4$ and HNO_3. Chufarov [4] holds that activation removes a surface film formed during heating, and which closes up the amorphous carbon atoms.

Recently, Barker [5] has attacked the problem with new technique. He finds that the distillation out of contact with air of wood or other source of carbon leads to the formation of a primary charcoal with a density of 1·45, and exhibiting no characteristic X-ray diffraction pattern. Upon activation, the density increases to a limiting value of about 2·15 and a characteristic X-ray pattern develops, resembling that shown by graphite in very small particles.

The increase in density of the charcoal leads to an internal shrinkage with consequent numerous small spaces within the granule, producing a crystalline surface. Accordingly, activation results in a more active surface, a greatly increased internal surface and a large volume of minute capillaries. Although during activation the proportion of hydrogen and oxygen decreased and that of carbon increased, in the best activated carbons both hydrogen and oxygen are present in considerable amount, and Barker considers that these components are present in unactivated or primary charcoal which serves as a binder to hold the granule firmly together.

Whilst the majority of workers define activity as the adsorptive efficiency of carbon under certain arbitrary conditions of tests, Lowry [6] points out that the adsorptive capacity may be raised "by any process which increases either the total surface per unit weight or the degree of unsaturation of the surface atoms, or both." Considering adsorption to arise from the forces due to unsaturated atoms in the surface,[7] a more satisfactory measure of activity would be the ratio : adsorptive capacity/total adsorbing surface. Thus, active carbon is defined as a carbon possessing a pronounced

[1] *J. Soc. Chem. Ind.*, **42,** 244 T (1923).
[2] *Z. physik. Chem.*, Abt. A., **149,** 211 (1930) ; *Kolloid-Z.*, **52,** 107 (1930).
[3] *J. Russ. Phys. Chem. Soc.*, **62,** 205 (1930).
[4] *Ibid.*, **62,** 883 (1930).
[5] *Ind. Eng. Chem.*, **22,** 926 (1930). *Vide* also Ogawa, *Biochem. Z.*, **161,** 275 (1925) ; **172,** 249 (1926).
[6] *J. Am. Chem. Soc.*, **46,** 824 (1924) ; *J. Phys. Chem.*, **29,** 1105 (1925) ; **32,** 1524 (1928) ; **34,** 63 (1930).
[7] Cf. Taylor, *J. Phys. Chem.*, **30,** 145 (1926) ; Gustaver, *Kolloidchem. Beihefte*, **15,** 185 (1922).

adsorptive capacity per *unit area* of adsorbing surface, rather than per unit eight. For two carbons of which the capacities for adsorption per unit weight are equal the one with least surface would possess the greater activity. On such views, according to Lowry, " an activation process would be limited to a process which increases the degree of unsaturation of the surface atoms, and would not include a process which simply increased the adsorbing surface." In defining the activity of a sample of carbon, therefore, not only should the adsorptive capacity be determined, but also the surface area (or pore volume),[1] which may be evaluated by a method based on heat of wetting and adhesion tension.[2]

REMOVAL OF COLOUR FROM SOLUTIONS

Chaney, Ray and St. John [3] point out two special cases in regard to colour removal from solution :—

(A) Provided the porosity of the carbon exceeds certain limits, its decolorising efficiency (adsorptive power) is completely defined by its activity, and is not related to the dimensions of the particles of the substance adsorbed.

(B) The adsorptive power of carbon for larger particles of colloidal dimensions is a function of its activity and of its apparent density and fineness, if its porosity is limited.

Whenever the substances to be adsorbed are of molecular dimensions, the activity as measured by the iodine test is regarded as a true index of its performance. With colloidal substances, however, " the adsorptive power of carbon is a mathematical function of the ratio of its activity to its apparent density, fineness being constant," *i.e.*, for carbons of equivalent porosity or apparent density, their power to adsorb colloidal particles is directly proportional to their activities. Again, it is emphasised that a carbon will adsorb particles better if its own electric charge is opposite to that of the material to be adsorbed.[4]

THE DECOLORISING OF SUGAR SOLUTIONS

As an introduction to this subject the student should read the paper by Blowski and Bon.[5] These workers found that the adsorption of colour by carbon was very rapid, approximate equilibrium obtaining after 15 minutes contact between carbon and the

[1] *Vide* Garner, McKie and Knight, *J. Phys. Chem.*, **31,** 641 (1927) ; Paneth and Radu, *Ber.*, **57,** 1221 (1924) ; Gustaver, *loc. cit.* ; Engel, *Z. ges. Schiess-Sprengstoffw.*, **24,** 451, 495 (1929).

[2] Bartell and Fu, *Am. Coll. Symp. Monog.*, **7,** 135 (1930).

[3] *Ind. Eng. Chem.*, **15,** 1252 (1923).

[4] Cf. Brewster and Raines, *Ind. Eng. Chem.*, **13,** 1043 (1921) ; Tanner, *ibid.*, **14,** 441 (1922).

[5] *Ind. Eng. Chem.*, **18,** 32 (1926). For technical details regarding the use of char, see Fairrie, " Sugar " (Liverpool, 1925), 79 *seq.*, 114 *seq.*

sugar solution. The colour removal followed the adsorption isotherm, and the need to work with dilute solutions is emphasised. They advocate the working at as high a temperature as possible between 40°—100° C., as the colour adsorption varies with the temperature, but below 40° C. such linear relation ceases, and adsorption is less as the temperature falls.

The reaction of the solution plays a *rôle* and decolorisation is better effected in an acid medium, although satisfactory decolorisation takes place "within the accepted safe alkalinity limits of refinery practice (pH 6·5—7·5)." The major portion of the colouring matter of washed raw sugar liquor (probably of colloidal dimensions) is readily adsorbed by a small amount of carbon, but there is a small residue of colour which is very difficult to adsorb.

All the carbons used by these authors removed ash to some extent, and this effect was enhanced by the fine-grinding of the carbons. There is evidence that the mineral skeleton of the carbon is responsible for this effect. However, since only small amounts of carbon are used and then usually with only high-purity liquors, the ash removed has little significance on the technical scale.

FACTORS INFLUENCING ADSORPTION

Within recent years numerous investigators have studied the various factors which influence the adsorption of colour by carbon from sugar liquors. Bradley [1] employed Norit and first of all investigated the influence of the size of the char particles. He found that up to 84—94 mesh, the adsorption of colour increases rapidly the smaller the carbon particles, but this grain-size effect disappeared after about 94 mesh. Coates [2] reported similar results with his Bagasse chars.

With respect to the amount of carbon to be employed, Bradley obtained the following results :—

Carbon Used.	Colour Adsorbed.	Filtrate.
Per cent.	Per cent.	
0·5	36·5	Not entirely bright.
1·0	62·5	,, ,, ,,
1·5	70·0	,, ,, ,,
2·0	75·4	,, ,, ,,
2·5	79·0	,, ,, ,,
3·0	82·7	,, ,, ,,
4·0	85·5	Bright.
7·0	88·0	,,

[1] *J. Soc. Chem. Ind.*, **38**, 396 T (1919).
[2] *Ind. Eng. Chem.*, **14**, 197 (1922).

Blowski and Bon's data [1] confirm these findings, colour removal not being linearly related to the amount of carbon used, but following the adsorption isotherm. Bradley believes that his results indicate that "the greater proportion of colouring matter is in a crude colloid state of considerable molecular dimensions and is, therefore, the more easily removed, whereas the last traces being much nearer a true solution are much more difficult to adsorb." [2]

As regards the influence of temperature, the following results were obtained when equal weights of 50 per cent. syrup with 5 per cent. carbon (calculated on basis of sugar solids) were agitated for 1 minute at different temperatures and then filtered :—

Temperature.	Per Cent. Colour Removed.
20° C.	54·60
30° C.	60·80
40° C.	64·50
50° C.	67·20
60° C.	69·72
70° C.	70·13
80° C.	71·11
90° C.	71·60
100° C.	71·95

The influence of the concentration of sugar in the solution was now investigated. As the sugar increases up to a concentration of 40 per cent. the decolorising efficiency rapidly falls, and thereafter falls much more slowly as the sugar concentration increases to 70 per cent. Thus, using a dark syrup and adding sugar, the following data were obtained :—

Sugar Added.	Colour Adsorbed.
Nil.	50·0 per cent.
10 per cent.	46·75 ,,
15 ,,	43·62 ,,
20 ,,	38·32 ,,
25 ,,	32·03 ,,
30 ,,	21·91 ,,
40 ,,	19·96 ,,
50 ,,	17·82 ,,
60 ,,	16·10 ,,

[1] *Loc. cit.*
[2] See Blowski and Bon, *loc. cit.*, p. 34, for a similar idea.

Comparable results confirming the above have also been published by Honig [1], and support the advice that one should employ diluted sugar liquors in decolorising processes.[2] According to Zerban [3] there is no adsorption of the sugar. Although Weinrich [4] asserts that vegetable chars do not remove organic and mineral impurities in addition to colour, whilst bone char does, Sauer [5] and others [6] find removal of nitrogenous substances, gums and ash constituents.

Confirming Blowski and Bon,[7] Bradley found an increase in decolorising efficiency directly proportional to the acidity, the nature of the acid making very little difference in the results. The importance of conducting tests under standard conditions of acidity has been stressed by Coates.[8]

EFFECT OF pH ON COLOUR ADSORPTION

Wijnberg [9] found that Norit removes the colour from cane juice better in slightly acid medium. Brewster and Raines [10] confirm this and recommend for technical practice that the juice and carbon be heated to boiling, adding phosphoric acid to pH 4·0. After a short time for reaction to be complete, the juice is limed back to pH 6·5, so as to avoid inversion of sugar.

The effect of pH on adsorption by carbons has been studied in some detail by Hague and Willaman [11] who employed eight varieties of industrial carbons. They followed the adsorption of electronegative, electropositive, amphoteric and non-electrolyte substances [12] by the various chars, and the general results are expressed in Fig. 44, reproduced from their paper. Caramel and benzoazurin were the electronegative substances, methylene blue the electropositive, egg-albumin and gelatin the amphoteric, and dextrose the non-electrolyte. It was observed that the pH value prevailing was of prime importance. Thus, the order of efficiency in decolorising at pH 7 was : Norit > Darco > Superfiltchar, > Carbrox > Apple char > Bone black > Blood charcoal and sugar charcoal, but at pH 4 the order was : Bone black > Norit > Apple char > Superfiltchar > Blood charcoal and sugar charcoal. It is of interest to note that while the adsorption of dextrose is not influenced by pH,

[1] *Kolloidchem. Beihefte*, **22,** 401 (1926).

[2] Cf. Blowski and Bon, *Ind. Eng. Chem.*, **18,** 32 (1926) ; Vašátko, *Z. Zuckerind. cechoslovak. Rep.*, **52,** 45 (1927) ; Linsbauer, *ibid.*, **51,** 483 (1927) ; Spengler and Landt, *Z. Ver. deut. Zuckerind.*, **77,** 429 (1927).

[3] Louisiana Agr. Exp. Sta., *Bull.*, **161,** 11 (1918).

[4] *Intern. Sugar J.*, **19,** 406 (1917).

[5] *Ibid.*, **20,** 24 (1918).

[6] *Loc. cit.*, p. 38.

[7] *Loc. cit.*, p. 33.

[8] *Ind. Eng. Chem.*, **14,** 296 (1922).

[9] *Intern. Sugar J.*, **17,** 50 (1915).

[10] *Ind. Eng. Chem.*, **13,** 1043 (1921) ; *Louisiana Planter*, **69,** 167 (1922) ; U.S. Pat. 1,447,461 (1923).

[11] *Ind. Eng. Chem.*, **19,** 943 (1927).

[12] Cf. Traube, *Z. Ver. deut. Zuckerind.*, **77,** 355 (1927).

alkalinity favours the adsorption of the electropositive methylene blue and " the adsorption of amphoteric substances, such as proteins, is at a maximum in the general region of the isoelectric point of the protein, and is at decided minima in extremes of acidity and alkalinity."

Active carbon may be charged in suspension and behave as electronegative or electropositive suspensoids, the charge arising from the adsorption of hydroxyl or hydrogen ions respectively.[1] Obviously, the negatively charged colouring matters in sugar

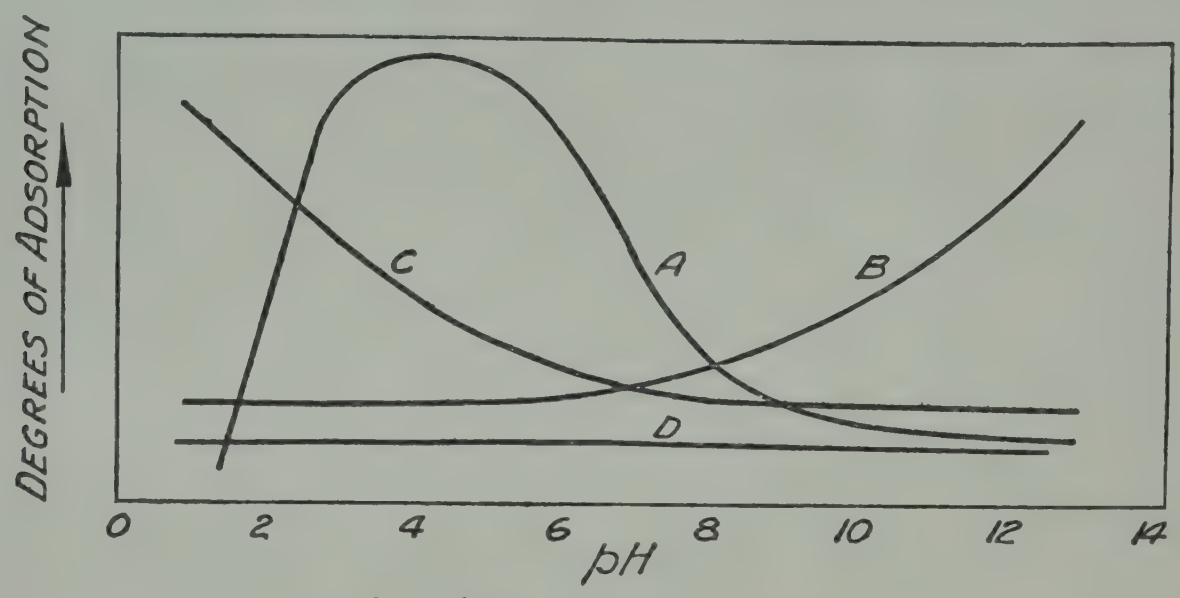


FIG. 44.—Generalised curves showing relation between hydrogen ion concentration and adsorption by chars of substances having various electrical properties.

solutions will be better adsorbed when the carbon particles are positively charged, *i.e.*, when in an acid medium.[2]

THE ADSORPTION OF SUGAR

Although there are statements to the contrary, there is now good evidence that during the decolorising of sugar solutions by carbons, sucrose is also adsorbed.[3] Vašátko [4] found the sucrose adsorption was primarily related to the surface area of the carbon and was always accompanied by adsorption of water. Increase in temperature decreases the adsorption of sucrose.

THE ADSORPTION OF ASH

Recent work has been directed to estimating the extent of the adsorption of ash constituents during the decolorising of sugar

[1] Cameron, *J. Phys. Chem.*, **14,** 400 (1910) ; Rona and Michaelis, *Biochem. Z.*, **32,** 85 (1919) ; Honig, *Kolloidchem. Beihefte,* **22,** 381 (1926) ; Sneller, *Louisiana Planter,* **59,** 154 (1917).

[2] For an excellent illustration of this fact see Chaney *et al.*, *Ind. Eng. Chem.*, **15,** 1254 (1923) ; cf. also Honig, *loc. cit.*, p. 400.

[3] *Z. Zuckerind. cechoslovak. Rep.*, **52,** 21 (1927).

[4] Kolthoff, *Biochem. Z.*, **168,** 122 (1926) ; Vacha, *Z. Zuckerind. cechoslovak. Rep.*, **55,** 123 (1930).

solutions by carbons. Dědek and Tumova [1] filtered thick beet juice and melted raw beet-sugar through layers of various thickness of washed and dried carbons. Specific effects were noted in respect to the adsorption of colour, nitrogenous matter and ash (CaO). Thus, the order of efficiency for removal of colour was : Carboraffin > Norit > Bone char ; for nitrogen : Norit > Carboraffin > Bone char ; and for CaO : Bone char > Carboraffin > Norit.

Sazavsky [2] confirms the order for CaO removal and states that for direct manufacture of white sugar from beet, one should use thick juice bone char. In his opinion, the phosphatic mineral skeleton adsorbs colouring matter and also is the only cause of the adsorption of the calcium ion. Although neutral, completely-washed, active carbons do not adsorb combined calcium, addition of $Ca_3(PO_4)_2$ or $CaHPO_4$ to such carbons causes them to behave like bone char.

Wayne [3] finds that " the seat of the ash-adsorbing powers is in the mineral structure of the char " and emphasises that " the presence of an adsorbed layer of electrolytes on the surface of charcoal greatly determines the type of adsorption occurring and largely determines whether it will be positive or negative for bases or acids and molecular or ionic in the case of neutral salts." He proved that the adsorption of ash from sugar solutions is greatly influenced by the presence of adsorbed alkaline [4] substances within the char, and, therefore, washed chars adsorb less ash.

Using a raw sugar solution of 50° Brix, filtered with filter-cel present, and taking 50 gr. char to 200 c.c. of sugar solution, he examined the filtrate after the mixture had stood at 80° C. for 1 hour. The ash content of the sugar solution was 0·240 per cent., and the extent of ash adsorption showed how marked is the influence of the previous treatment of the char. The ash removed varied from 5·4 per cent. to 47·5 per cent., and in two instances the char lost ash to the solution.

THE ADSORPTION OF CARAMEL

The adsorption of caramel has been investigated by Hauge and Willaman,[5] using Norit and other chars. They present a detailed table of results, wherein it is evident that pH has a pronounced influence. Adsorption is most successful in very acid medium and decreases as the pH increases. In fact, adsorption will only take

[1] *Z. Zuckerind. cechoslovak. Rep.*, **52,** 65 (1927).
[2] *Ibid.*, **52,** 13 (1927).
[3] *Ind. Eng. Chem.*, **20,** 933 (1928). *Vide* also, Knowles, *ibid.*, **19,** 222 (1927) ; Rice and Murray, *ibid.*, **19,** 214 (1927) and **20,** 276 (1928) ; Wijnberg, *Intern. Sugar J.*, **18,** 194 (1916) ; Weinrich, *ibid.*, **20,** 424 (1918) ; Sauer, *ibid.*, **20,** 24 (1918) ; Avot, *ibid.*, **25,** 196 (1923) ; Zerban and Taggart, Louisiana Agr. Exp. Sta., *Bull.*, **161** (1918).
[4] Cf. Coates, *Ind. Eng. Chem.*, **14,** 295 (1922).
[5] *Ibid.*, **19,** 944 (1927).

place on sugar char and blood charcoal in acid solutions. Fig. 45 is reproduced from their paper, and the differences in the character of the curves reflect different characteristics of the chars employed.

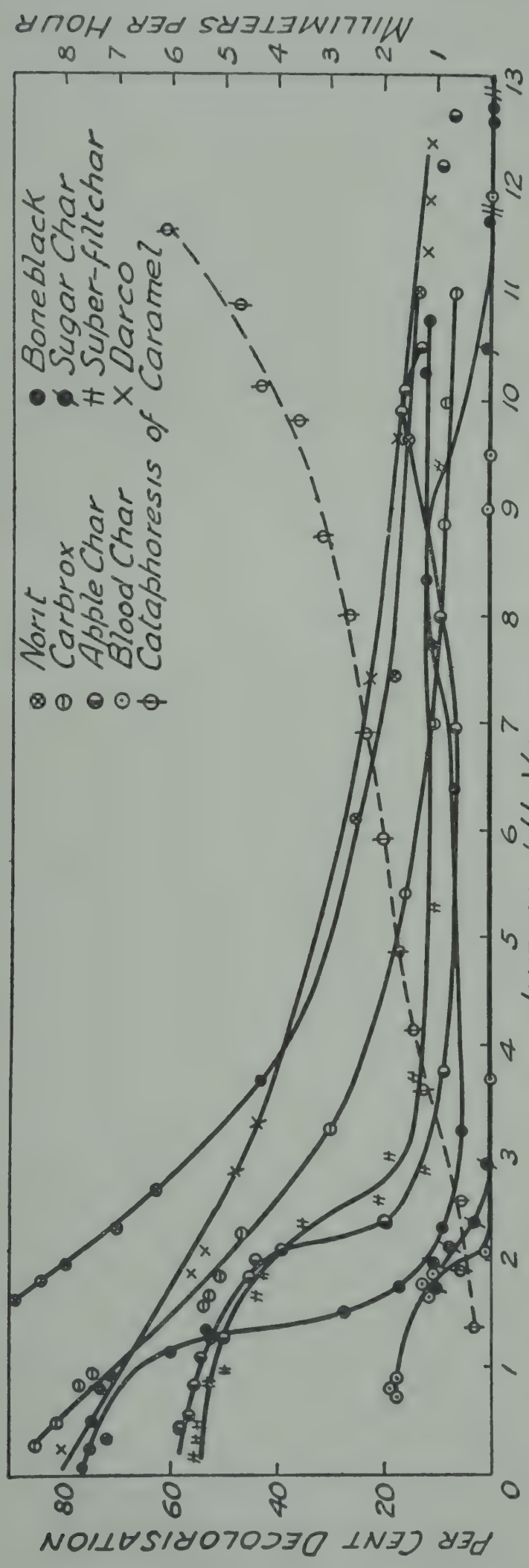


FIG. 45.—Adsorption of caramel by various chars at various hydrogen ion concentrations.

THE ADSORPTION OF COLLOIDS FROM MOLASSES

Brodowski[1] investigated the adsorption by Norit of the irreversible colloid A_3 (see p. 313) obtained from beet molasses. When 25 c.c. of a 0·5 per cent. solution of A_3 was treated with 0·25 gr. Norit at 70°—80° C. for 30 minutes, the percentage decolorisation in the filtrate was 34·6 and 29·2, when the original solutions were at pH 5·4 and 7·5 respectively, *i.e.*, there is more effective decolorisation in acid medium.

The reversible colloid C_4 was made into a 1 per cent. solution (pH = 5·5) and 25 c.c. treated with 0·25 gr. of various chars. It was noted that Carboraffin was superior to Norit, the decolorising effects being 52·8 per cent. and 29·1 per cent. respectively. The superiority of Carboraffin is attributed to its acid character and the close proximity of the pH of the solution (pH 5·3) to the isoelectric point of the colloid.

FILTERING EARTHS

As a preliminary to the clarification of sugar liquors by carbon various inorganic filtration or decolorising earths have been proposed.

[1] *Kolloidchem, Beihefte,* **29,** 324 (1929).

such as kieselguhr,[1] bauxite [2] and filter-cel.[3] a diatomaceous earth. The purpose of such earths is to remove colloidal matter. An interesting example of the application of earths has been outlined by Paine and Badollet.[4] In the course of the manufacture of corn syrup and corn sugar, the acidity of the acid starch conversion liquor is reduced by means of Na_2CO_3, and this results in the flocculation of some of the colloidal matter due to pH change. However, even at the isoelectric point all the colloids are not removed, and the clear filtrate will gradually become turbid. Now the colloids in the very acid conversion liquor are *positively* charged, so that addition of a colloid which would still carry a negative charge under such acid conditions should prove highly effective in causing the sugar colloids to become flocculated.

Bentonite, a colloidal clay, was used with marked success. When added to acid converter liquor until the isoelectric point of the colloids was reached, as shown in a cataphoresis cell under the ultramicroscope, copious flocs resulted which readily settled out. The clear liquor could now be reduced in acidity by adding Na_2CO_3, but the pH value had to be less than 5 in order to retain brilliancy.

HONEY COLLOIDS

Certain dark-coloured, low-grade honeys must necessarily be used only in confectionery, but even then the low caramelisation point is a defect. Lothrop and Paine [5] have recently examined such honey, particularly buckwheat honey, from the colloid-chemical standpoint. By ultrafiltration they ascertained that the colloid content of various honeys ranged from about 0·1 per cent. in the case of some light honeys to about 1 per cent. in dark samples, such as buckwheat.

Dye tests and cataphoresis demonstrated that the colloid matter in most honeys carries a positive electric charge, in sharp contradistinction to sugar colloids. At pH $4{\cdot}3 \pm 0{\cdot}1$ the isoelectric point is sharply defined. Although at this pH value turbidity soon manifests itself, several hours must elapse for flocculation and settling to occur.

A few honeys showed their colloid content to be negatively charged; these were of the honeydew type and characterised by comparatively high pH values and high ash content which exerted an increased buffer effect on the acids present. Such honeys possessed pH values in the range 5·12—4·38 whilst the others had pH values in the range 4·30—3·57. However, with all the honeys examined, the isoelectric point was in the pH range 4·00—4·43.

[1] Paine and Walton, *Science*, **53**, 266 (1921).
[2] Devos, *Bull. assoc. chim. sucr. dist.*, **46**, 292 (1929).
[3] Elsenbast *et al.*, *Ind. Eng. Chem.*, **21**, 676 (1929).
[4] *Facts about Sugar*, **21**, 1212 (1926); *Ind. Eng. Chem.*, **19**, 1245 (1927).
[5] *Ind. Eng. Chem.*, **23**, 328 (1931).

Using 50 c.c. portions of 25 per cent. honey solutions and adjusting the pH value by means of 0·1 N. HCl or NaOH, the authors determined the quantity of flocculated colloid matter at different pH values and furnish the following table illustrating their results.

TABLE 64. VARIATION OF WEIGHT OF FLOCCULATED COLLOIDS WITH pH.

Clover Honey.			Amber Alfalfa Honey.			Buckwheat Honey.		
pH.	Charge on Colloids.	Weight of Flocculated Colloids.	pH.	Charge on Colloids.	Weight of Flocculated Colloids.	pH.	Charge on Colloids.	Weight of Flocculated Colloids.
		Mg.			Mg.			Mg.
2·78	+	11·8	3·12	+	19·4	3·21	+	22·4
3·02	+	12·1	3·38	+	21·0	3·40	+	26·6
3·57	+	14·7	3·74	+	22·9	3·69	+	34·2
4·03	+	18·6	3·98	+	24·0	3·98	+	68·2
4·39	Neutral	19·0	4·31	Neutral	24·2	4·32	Neutral	98·7
4·62	—	18·6	4·62	—	18·0	4·67	—	55·6
5·13	—	13·1	4·91	—	14·8	4·96	—	19·7
6·05	—	9·3	5·27	—	10·8	5·13	—	4·9

Ultrafiltration is not a commercially practicable method of removing colloid matter from honey, but the use of bentonite (negatively charged particles) brought about the mutual flocculation of the colloids. Unfortunately, such treatment involves previous dilution of the honey with water, and its subsequent removal by evaporation from the filtered colloid-free honey.

The bentonite treatment resulted in honeys of higher pH value, higher surface tensions, lower titratable acidities and slightly higher ash contents. The colour was improved and the flavours were milder.

The authors mention that apart from colloid matter which is flocculated at the isoelectric point or by addition of bentonite, certain honey colloids remain unflocculated, being stabilised primarily by hydration.

CHAPTER X

FRUIT JELLIES, JAMS AND MARMALADES

Of great importance in the diet are jellied products prepared from fruit juices and sugar, such as jams, marmalades and various fruit jellies. Questions of much interest to the colloid chemist abound, mainly involving problems of optimum physico-chemical factors, jelly strength, syneresis, etc.

The fundamental study concerns the system pectin : aqueous solution of acid : sugar, and a mass of published work deals with the matter. Jelly formation results only over a definite equilibrium range of the system, and is affected by such factors as temperature and ion concentrations.

It is well-known that certain fruits readily make into fruit jellies or jams, their content of pectin and acid being sufficiently high. Others do not form jellies although they may be rich in pectin or acid, but not in both. The following table illustrates this.

Jellying Fruits.	Non-Jellying Fruits.	
Pectin and Acid Sufficient.	Pectin-rich.	Acid-rich.
Sour apples, red currants, gooseberries, strawberries and raspberries (not too ripe) ; acid plums, sour quinces, lemons, longberries, oranges, limes.	Sweet apples, peaches, pears, figs.	Rhubarb, tomatoes.

PECTIN

Since the discovery of jelly-forming substances in fruits by Braconnot [1] and the early important researches of Frèmy,[2] the nomenclature of pectin and related compounds has varied so much as to be somewhat confusing. Thus becomes all the more valuable the recent summary of definitions by a Committee on Nomenclature of Pectin of the American Agriculture—Food Division.[3] The recommended definitions read as follows :—

DEFINITIONS WRITTEN BY THE COMMITTEE ON NOMENCLATURE OF PECTIN OF THE AGRICULTURE-FOOD DIVISION

Pectic Substances. A group designation for those complex carbohydrate derivatives which occur in plants, or are prepared from

[1] *Ann.*, **5**, 275 (1833).
[2] *J. pharm. chim.*, **26**, 368 (1840).
[3] *J. Am. Chem. Soc.*, **49**, Proc. 37—9 (1927). Reprinted through the courtesy of the Editor of *The Journal of the American Chemical Society*.

plants and which are characterised by the presence of galacturonic acid units. In the naturally occurring pectic substances, the galacturonic acid units apparently exist in an acid-reacting complex associated with arabinose and galactose units. This acid complex may occur as a free acid or as a metallic salt, but usually occurs as a methyl ester.

Protopectin. The term applied to the water-insoluble, unhydrolysed pectin substances in the state in which they occur in plant tissues. *Protopectins* are rendered soluble by treatment with enzymes, acids or certain other reagents, whether by peptisation or hydrolysis not being known. The substances thus rendered soluble are designated collectively as *pectin* ; it is not known whether or not the *pectins* are different chemical individuals from *protopectins*.

Pectin. The term applied to the water-soluble, methylated pectin substances occurring in plant tissues or to the methylated pectic substances obtained by restricted treatment of *protopectin* with *protopectinase*, acids or other reagents, the treatment being so regulated as to produce maximum solution of pectic substances with a minimum cleavage of methyl ester groups. The product may be a mixture of substances of varying methyl ester content ; the term *pectin* or *pectins* is accordingly a group designation for all intermediate pectic substances between *protopectin* and *pectic acid*. It is proper, however, to refer to an individual of the group as a *pectin*.

Pectic Acid. The term applied to the pectic substances obtained by the hydrolysis of *pectin* with the complete elimination of the methyl ester groups. Pectic acid may be variable in composition according to the type of hydrolysis employed ; accordingly, there may be a number of *pectic acids*, all of which are ester-free.

Protopectinase. The term applied to the enzyme which hydrolyses or dissolves *protopectin* with the resultant separation of the plant cells from each other, usually spoken of as maceration. Presumably the product of this hydrolysis is pectin. The term *protopectinase* supersedes the older term " pectosinase " with which it is synonymous.

Pectase. The term applied to the enzyme which converts *pectin* into *pectic acid*, the latter becoming a gel, especially in the presence of calcium (or barium or strontium) salts.

Pectinase. The term applied to the enzyme which hydrolyses *pectin* and *pectic acid* into their simplest soluble cleavage products, which are probably arabinose, galactose and galacturonic acid.

Statement [1] to Accompany Report of the Committee on Pectin Nomenclature

The nomenclature for the pectic substances was arrived at after considerable discussion and correspondence. Since similar discus-

[1] This statement extends to the end of paragraph 5 on p. 343.

sions are likely to arise again, it appears desirable that a statement be made of the reasons which guided the committee in the wording of each of the definitions.

Pectic Substances. This name has been used in recent literature by von Fellenberg, Sucharipa, and others, and appears to be almost a satisfactory general designation. It appears undesirable that the term *pectin* should be retained as a loose general designation for all substances of this type ; this term should rather be restricted to the soluble substances defined under *pectin*, thus obviating the need of the expedient so frequently used in the past, of referring to *soluble pectin* in order to distinguish *pectin* from *protopectin*.

The substances are designated as " carbohydrate derivatives " in contradistinction from " carbohydrates." Pectic substances are distinguished from polysaccharides in general by the possession of carboxyl groups. These carboxyl groups are now known to reside in sugar acid units which presumably originate by the oxidation of alcoholic groups of sugar units. Accordingly it appears consistent with the physiological origin of pectic substances, as well as with their chemistry, to designate them as carbohydrate derivatives.

As far as is known, galacturonic acid units are the only acid units found in pectic substances. It is true that further studies may disclose the presence of other acids and it may prove necessary at a later date to make the definition more general by substituting " hexuronic acid " for " galacturonic acid." Such a generalisation appears undesirable in view of the limits of our present knowledge.

The present definition of the pectic substances is intended to confine the term to the gelatinisable substances of the types that are found in fruits, in the succulent edible vegetables, and in cell wall tissues generally. It is intended to exclude plant muci ages, algin-like substances from marine algae, cactus slimes, etc., although these bodies are apparently closely related, chemically, to the pectic substances. From the meager data available, it appears that these substances contain other acid groups than galacturonic acid ; for example, Sven Oden reports the presence of a pentonic acid in a preparation from marine algae, and Spoehr asserts that glucuronic acid occurs in cactus. The definition of pectic substances as substances containing galacturonic acid appears to effect the desired distinction from these other classes of bodies.

As far as the committee is aware, all naturally occurring pectic substances contain arabinose and galactose units but in the case of laboratory preparations, the carbohydrate units of the original pectic substance may be partly or wholly split off by the hydrolytic treatment employed. The definition of pectic substances has been worded so as to recognise these facts.

Protopectin. The original pectic substance has been variously designated as *pectose*, as *protopectin*, and as *pectinogen*. Pectose was

named by Frèmy in 1840, protopectin by Tschirch in 1908, and pectinogen by Schryver and Haynes in 1914. The name pectose accordingly takes priority and is strongly established in the literature of plant physiology. Chemically, it is an unfortunate term, since the suffix *ose* is almost exclusively reserved for the crystalline sugars (with one important exception—cellulose—which is apparently too firmly established to be changed). Since *protopectin* was proposed by Tschirch in 1908, it has been accepted and used by von Fellenberg, Neuberg, Ehrlich, Sucharipa, and in fact, by practically all pectin chemists of continental Europe. A canvas of American pectin chemists indicates that nearly all of them prefer this term. Some English investigators, on the other hand, prefer and use *pectinogen*, and one American worker has expressed a preference for this term on etymological grounds. Although the derivation would suggest that it refers to the original pectic substance, and although it has been so used, the term pectinogen was created to apply to a soluble pectin isolated by a definite type of treatment. In view of the fact that this term has been used in a dual sense whereby confusion has arisen, and of the fact that the term protopectin takes priority and is already better established, the selection of protopectin as the designation for the original pectic substance appears logical and satisfactory. The term pectinogen is legitimately applied to a soluble pectin prepared by the treatment described by Schryver and Haynes.

Pure protopectin has never been isolated for the reason that it has no known solvents and therefore cannot yet be separated from the accompanying and likewise insoluble cell-wall tissue. Nevertheless, the existence of this substance has been assumed ever since pectic substances were known. From the results of investigations on apple pomace and unripe apples, which appeared to indicate that all of the pectic substances in these materials are water-soluble, Tutin concluded that protopectin has no real existence. It is to be noted that Tutin's investigations were confined to a single type of material, and that, even as applied to his material, his conclusions have been questioned by Carre. Sucharipa, on the other hand, has brought forward direct evidence which apparently indicates that the insoluble protopectin is a pectic substance in combination with cellulose, and that the soluble form is obtainable only after separation from the cellulose by hydrolytic treatment. The committee's conception of the nature of protopectin rests to a large extent upon the evidence submitted by Sucharipa, which in our opinion, is sufficient to justify the recognition of protopectin as a distinct class of pectic substances.

A different view has been recently advanced concerning the nature of the original pectic substance found in the cell walls of plants. According to this view the substance which has been designated as protopectin, is regarded as essentially the same

substance as pectin. The proponents of this view reject the conception that the conversion of insoluble pectic substance to pectin under the action of acids or other reagents is necessarily a process of hydrolysis ; it is argued that possibly the original undissolved pectin is so strongly adsorbed by the cell-walls, that it cannot be extracted with water whereas it can be extracted by acid. On the basis of this viewpoint, it is contended that evidence is lacking for the existence of protopectin as a separate substance differing from pectin.

It is the opinion of the committee on Pectin Nomenclature that the traditional view, *i.e.*, that the conversion of the original undissolved pectic substance into dissolved pectin is a process of hydrolysis, is the simplest explanation of the observed facts ; furthermore, that there is a considerable amount of experimental evidence in support of this traditional view. Strong support for this view is found in Sucharipa's observation that, during treatments which convert the undissolved pectin of plants into soluble forms, a quantity of cellulose is simultaneously rendered soluble in ammoniacal copper oxide solution, and this in regular proportion to the quantity of pectin dissolved. The behavior of plant tissues containing insoluble pectic substances when subjected to the action of enzymes, is especially consistent with the traditional view. On the other hand, it must be admitted that the conception of protopectin as a colloidally adsorbed pectin may be the correct one. However, until evidence is forthcoming which will definitely indicate that protopectin has no real existence as a substance distinct from pectin investigators will continue to require some term to describe the undissolved pectic substance of the cell wall of plants. Accordingly, it appears to the committee that the definition of protopectin as a separate substance should be retained, but expressed in such terms as will indicate that possibly it may be chemically the same as the soluble pectins.

Pectin. The definition here given is in conformity with the traditional use of the term in its more restricted sense. There has been some question as to the advisability of including in this class those members of the series that are low in methyl ester content and apparently not useful in jelly formation. It has been suggested that since they approach pectic acid in commercial value, it might be convenient and satisfactory to classify the slightly methylated pectins with the pectic acids, especially since they tend to show acid characteristics. There are two objections to such a classification : (1) the classification, to be scientific, should be based upon chemical composition or some other accurately ascertainable characteristic, rather than upon commercial value, and (2) it would be very difficult to determine where to draw the line between pectin and pectic acid. Accordingly, the chemical classification has been retained and the presence of methyl ester content is assumed to characterise pectins

and to distinguish them from pectic acids which are consequently assumed to be free from methyl ester. (This classification has been used by Sucharipa in his recent monograph, " Die Pektinstoffe," Serger & Hempel, Braunschweig, Germany. See p. 95.)

In stating the type of hydrolysis that produced pectin from protopectin, it is not the intention of the committee to define pectin as the product of a treatment. The defining characteristics of pectins are their methylated condition and (with the possible exception of some slightly methylated pectins), their water-solubility ; the phrase " restricted hydrolysis " is introduced to distinguish the preparative treatment of pectins from treatments that would result in pectic acids.

Sucharipa classifies the pectins into (1) *free pectin*, occurring as such in the plant and removable by simple water solution, and (2) *hydrolysis pectin*, resulting from protopectin by treatment with hydrolysing agents. Chemically, they are distinguished by the fact that the free pectin retains arabinose units while the hydrolysis pectins do not, the pentose units having split off during the hydrolysis. (See citation above.)

The classification offered by the committee affords opportunity for later subdividing the pectins as proposed by Sucharipa, or in other ways. The committee does not consider it advisable to recommend any subdivision of these main groups at present. The committee regards its present task to be the clarification of the general terms used with references to the pectic substances, and the grouping of the various recognised pectic substances into their general classes on the basis of their chemical similarity. Once this is accomplished, it will be comparatively easy to decide upon the further subdivision of the groups and to fit individual substances into the general scheme.

Pectic Acid. Doubt has been expressed as to whether all substances heretofore classified as pectic acids are completely free from methyl ester. If the classification is to have a sound chemical foundation, it appears necessary to assume that we have a sharp separation into *pectins* which are esters and completely de-esterified *pectic acids*. The reasons have already been discussed under the section entitled *pectin*. Further researches may, of course, necessitate reclassification of some of the substances now known as pectic acids, since, if it is found that they still retain methyl ester groups, they will then be regarded under the definition as acid esters, or *pectins*.

The methyl-ester-free pectic acids are probably variable in composition according to the amount and kind of non-methoxyl groups (*i.e.*, arabinose, galatose, etc.) that are retained with them. According to the type of hydrolysis by which they are formed, we may expect to find the groups of the original pectin split off or

retained in varying proportion. The pectic acid contained in the calcium pectate of Carre and Haynes, the cytopectic acid of Clayson, Norris and Schryver, and the pectic acid of Wichmann and Chernoff are all regarded as pectic acids under the definition given, but they probably differ in composition as they differ in preparative treatment.

Protopectinase. In order to conform to the term protopectin, it appears desirable to discontinue the traditional name *pectosinase* for the enzyme which acts upon it, and to substitute the new term *protopectinase.* Although the older term is well established in the literature, it is believed that the introduction of a new term will not cause confusion as the naming of enzyme and substrate follows the usual convention and the significance of the term is self-evident.

Pectase. In this definition is retained the significance that has been attached to the term ever since it was first stated by Frèmy. Mention of gel formation gives expression to the recognised coagulating effect of the enzyme.

Pectinase. This definition also is the traditional one given by Frèmy. It appears to the committee desirable that these definitions be restated at this time in essentially their original forms.

It has been suggested to the committee that recommendations be made that the terms *metapectin* and *parapectin* be dropped. The committee is of the opinion that these terms are already obsolete and that no useful purpose will be served by making any formal statement concerning them.

Valuable work on pectin bodies was published in 1918 by von Fellenberg,[1] who recognised three groups : (*a*) proto-pectin (Frèmy's pectose), (*b*) pectin, (*c*) pectic acid. Some important facts concerning these groups are summarised in the following table :—

Protopectin.	Pectin.	Pectic Acid.
In succulent root vegetables and unripe fruits.	In ripe fruits.	In unripe fruits and decayed vegetable tissue.
Insoluble in water.	Soluble in water. Insoluble in alcohol.	A weak acid. Prepared by dissolving pectin in dilute NaOH and adding HCl.
No jelly with sugar.	Is the active jellying principle.	Only gels with sugar when pectase is present.

Pectin is present chiefly in fruit and vegetable pulp, mainly in the insoluble carbohydrate portion or " marc." Little is present in

[1] *Biochem. Z.*, **85,** 118 (1918).

the juice. Thus, apple skins, apple pomace, inner rinds of lemons and oranges, beet pulp [1] and carrots are good sources of pectin. Wilson [2] gives the following data regarding the percentage of pectin in certain raw materials :—

Material.	Per Cent. Pectin in Fresh Material.	Per Cent. Pectin on Water-free Basis.
Apple pomace . . .	1·5—2·5	15—18
Lemon pulp . . .	2·5—4·0	30—35
Orange pulp . . .	3·5—5·5	30—40
Beet pulp . . .	1·0	25—30
Carrots	0·62	7·14

In preparing pectin the general principle is to remove solutes with cold water (as by soaking overnight), so that extraction with hot water will result in a purer pectin. Evaporation of the filtered solution leads to a syrup or powder, as desired.

As typical of the preparation of pectin for commercial purposes we may consider the work of Poore [3] on citrus pectin. In the thousands of tons of lemon, orange and grape-fruit residues as peel, pulp and seeds, the American citrus products factories have an enormous potential source of pectin, the raw material containing from 1·5 to 3 per cent. of pectin depending on the ripeness of the fruits.

Lemon peel is heated in 0·4 per cent. aqueous citric acid at 98° C. for 1 hour, filtered, and again extracted with a fresh acid solution at 98° C. for 1 hour. Further extractions produce poor yields of inferior pectin. The combined extracts are clarified with kieselguhr. The bitter principles of lemon must now be removed. The pectin solution is concentrated at 60° C. to a thick paste containing from 25 to 50 per cent. total solids. Addition of alcohol [4] removes the bitter principles and leads to a granular non-gelatinous mass, the alcohol content of which can be removed by pressure, followed by drying (below 70° C.).

The fact that extraction with dilute acids gives a greater pectin yield and a more effective jellying pectin has been emphasised by Johnstin and Denton,[5] who also pointed out that autoclave extraction is not commercially feasible owing to the rapid hydrolysis of pectin. Thus citrus pectin extracts obtained by autoclaving at 108° C. (5 lbs. pressure) for 30 minutes had only 75 per cent. of the jellying power of similar extracts prepared in open kettles.

[1] Wohryzek, " Chemie der Zuckerindustrie " (Berlin, 1928), p. 43—58 ; Codling and Woodman, *J. Agr. Sci.*, **19**, 701 (1929).
[2] *Ind. Eng. Chem.*, **17**, 1066 (1925).
[3] U.S. Dept. Agr., *Dept. Bull.*, **1323** (1926).
[4] Cf. Bourquelot and Herissey, *J. pharm. chim.*, **7**, 473 (1898).
[5] *Ind. Eng. Chem.*, **15**, 778 (1923) ; cf. Mehlitz, *Z. tech. Biol.*, **11**, 134 (1925).

Recently, Wilson [1] has described the commercial preparation of pectin from lemon refuse (from citric acid manufacture). Unless the lemon refuse is to undergo prompt factory treatment, it is heated to 95°—98° C. for 10 minutes to destroy pectase, which would cause the break down of pectin to pectic acid.

In usual practice [2] the disintegrated peel is washed and a charge of 500—1,000 kg. run into wooden tanks fitted with false bottoms covered with filter cloth. The mass is suspended in 1 per cent. aqueous sulphurous acid, brought rapidly to 90° C. and maintained thereat for 2 hours. The liquor is drained off and a second or third extraction made. The combined extracts are filtered and the pectin solution (about 0·5 per cent. strength) quickly cooled. The excess SO_2 is removed by passing the solution over wooden baffles as in water-cooling towers. The usual charge of 5,000 litres of solution is run into a 7,500 litre tank.

Precipitation of the pectin (a negatively-charged colloid) is effected by means of colloidal aluminium hydroxide [3] (a positively-charged colloid). The exact quantities are determined by a laboratory test on a sample from the batch, ammonia and aluminium sulphate being used to produce the necessary aluminium hydroxide. The ammonia is added to the agitated liquor and immediately afterwards the requisite aluminium sulphate, the latter as a 25 per cent. solution. Optimum precipitation results when the quantities of ammonia and aluminium sulphate are such that after precipitation, the supernatant liquor is at pH = 4·0 — 4·2.

Violent aeration of the suspension (*e.g.*, by agitation) floats the precipitate as a thick spongy foam. After filtration, the precipitate is washed in water and then centrifuged or pressed. The residue is dried at 65° C., giving a dark green horny mass which is now ground to pass 100 mesh. The ash content (mainly Al_2O_3) amounts to 10—12 per cent., and to effect its removal advantage is taken of the solubility of aluminium chloride in alcohol. The crude pectin powder is suspended in 85 per cent. alcohol containing about 10 per cent. by volume of concentrated hydrochloric acid. This stage is analytically controlled so that an excess of the acid is present. The pectin is washed with alcohol and dried at about 45° C. in a vacuum oven.

The final pectin, a grey powder, gives a jelly with sugar of neutral colour and taste, and is capable of jellying from 140 to 220 times its own weight of sucrose.

The problem of the extraction of pectin has recently been considered from quite a new standpoint, Myers and Baker [4] publishing results of decided scientific interest and practical importance. Their

[1] *Ind. Eng. Chem.*, **17**, 1065 (1925).

[2] See also Ajon, *Riv. ital. essenze profumi.*, **11**, 107 (1929); Leone, *Ann. chim. applicata.*, **20**, 433 (1930); Melis, *Giorn. chim. ind. applicata.*, **11**, 399 (1929).

[3] Cf. Magoon and Caldwell, *Science*, **47**, 592 (1918).

[4] Univ. Delaware Agr. Exp. Sta., *Bull.*, **160** (Tech. No. 10), 1—64 (1929).

aim was to establish the conditions whereby the most economical yield of pectin would result, this being definitely linked up with its jellying power. "The product of the yield times the jelly grade is designated by the term 'jelly units,' and is a measure of the quantity of jelly that may be obtained from any weight of albedo, since the quantity of jelly is proportional to the amount of added sugar."

As raw material was used the white part (albedo)[1] of lemons, carefully removed by hand and extracted with strong alcohol to remove resins, tannins, terpenes and glucosides. The preliminary investigation concerned the influence upon the *yield* and *quality* of the pectin of such factors as the time of boiling and the acidity of the water used. The important result was established that the jelly *grade* of a pectin reached an optimum value when the extraction was carried out with boiling water at a pH = 2·40, independent of the titratable acidity of the aqueous acid, and of the nature of the acid. More acid solution resulted in hydrolysis of the pectin with a consequent marked decrease in the jellying capacity of the pectin.

The duration of the boiling was also an important factor. Drawing a graph to show the relationship between the jellying power of the pectin and the duration of the boiling at constant pH value = 2·62, it was found that three distinct breaks occur in the curve, viz., at 15, 30 and 60 minutes' boiling. After 1 hour's boiling the jellying power is a linear function of the duration of boiling.

It was observed that after two hours boiling at pH = 2·62, no appreciable increase in yield resulted. "Approximately three times as much jelly may be obtained from the albedo, when extracting the pectin at a pH of 2·62, by boiling for 30 minutes, as by boiling three hours at the same hydrogen ion concentration."

The *yield* of pectin depended upon the pH during extraction, the maximum yields being obtained at pH = 2·0, using tartaric acid, and pH = 1·45, using HCl. The duration of boiling had an influence on yield less marked than on quality. A rapid increase in the yield of pectin followed boiling from 5—60 minutes, further boiling giving a less pronounced yield up to 120 minutes, after which no further extraction resulted.

Combining the data on quality and yield, the authors found that a pectin of maximum *jelly units* resulted when extraction of the pectic material was carried out at pH = 2·15, boiling for 30 minutes, a result independent of the nature of the acid used.[2] In this way, a pectic material will yield all its pectin in a single extraction. The practical performance of this method of extraction, based on a 25 lb. batch of pectic material, is described by Myers and Baker :—

Run 150 gallons of water, with an acid content adjusted so that

[1] For the complete analysis of lemon albedo, see Sucharipa, *J. Am. Chem. Soc.* **46,** 155 (1924).

[2] *Vide* Spencer, *J. Phys. Chem.*, **33,** 2015 (1929).

the extraction will proceed at pH 2·15, into a tank and heat to boiling. Add 25 lbs. pectic material (dry basis), *i.e.*, approx. 34 per cent. extractable pectin ; for material with less pectin, use less water. After boiling 30 minutes, run the mixture into a tank containing 100 gallons of cold water. Agitate and cool the mixture to below 30° C. The cooled liquor is then run through a filter press using filter-cel, and the clear filtrate is then available for the recovery of pectin by any desired method.

A detailed account of the technical preparation of apple pectin in liquid and in powder form has been given recently by Rooker,[1] to whom reference should be made.

Pectin is marketed as a syrup of about 4·5 per cent. concentration, or as a powder, described in " grades." Thus, " 100 grade pectin " refers to the fact that under the proper conditions of acidity, the pectin will make a jelly with 100 times its own weight of sucrose. The powder is made into solution for factory use, *e.g.*, in jam-making, by mixing with about 1/10th of the total sugar to be used in the final product. The pectin-sugar mixture is added to the necessary water or fruit-juice and stirred until all lumps are disintegrated. The solution is heated to boiling and the remainder of the sugar then added. Passing the solution through a colloid mill gives a really smooth gum-like solution.

COLLOIDAL PROPERTIES OF PECTIN

Pectin is a lyophilic colloid resembling gelatin or agar when in solution. It is difficult to dissolve in cold water, but yields aqueous solutions which are opalescent in reflected light and clear in transmitted light. Griggs and Johnstin [2] observed the Tyndall effect and Brownian motion. As already mentioned, pectin is negatively charged. It is readily precipitated by metallic ions, lead being particularly effective in setting the pectin to a hard, clear gel.

The dialysis of a 0·25 per cent. solution of lemon pectin in 0·01 N. hydrochloric acid in a parchment bag against water has been described by Griggs and Johnstin.[3] The water was heated once daily to 90° C. to prevent bacterial action. Acid was added daily to the pectin solution, until after about a week the outer liquid gave no further reaction for calcium. A further 4 or 5 days' dialysis removed the hydrochloric acid. The ash content of the pectin was thus reduced from 2·6 per cent. to 0·18 per cent., approximately 20 per cent. of the pectin being lost during dialysis.

The dialysed pectin sol readily yields pectin when 2 volumes of 95 per cent. alcohol are added by drops to the stirred aqueous solution. Alternatively, cataphoretic precipitation at the anode may

[1] " Fruit Pectin " (New York, 1928).
[2] *Ind. Eng. Chem.*, **18**, 623 (1926).
[3] *Loc. cit.* ; see also electrodialysis by Spencer, *J. Phys. Chem.*, **34**, 429 (1930), and Emmett, *Biochem. J.*, **20**, 564 (1926).

be obtained, using 110 volts, D.C. Several washings with alcohol, and finally with ether, gave a snow-white powder.

The same authors investigated the viscosity of aqueous pectin sols, using an Ostwald viscometer at 25° C. The following table shows the marked rise in viscosity with concentration :—

Pectin.	Specific Viscosity.
0·10 per cent.	1·348
0·25 ,, ...	1·834
0·50 ,, ...	2·899
0·65 ,, ...	4·018
0·80 ,, ...	5·190
1·00 ,, ...	7·235

Recently Mehlitz [1] has made important research into the colloid chemistry of pectin, treating it as a reversible hydratable (emulsoid) colloid. Dialysis of a pectin-sugar jelly gave a pectin sol. The investigation concerned the size of the pectin particles in the sols, and the relation between dispersion and jellying power. Mehlitz employed Bechold's method of fractional membrane filtration,[2] using a solution of apple pectin at pH = 3·05. The pectin content, expressed as calcium pectate was 3·44 per cent. It was found that the pectin particles in such a solution had the following dispersion range : one-third > 0·6 μ., and almost two-thirds between 0·2 μ—0·6 μ. Less than 10 per cent. of the total pectin had a particle size below 0·2 μ.

When an apple pectin solution at pH = 3·00 is submitted to fractional membrane filtration, as the pore-size of the filters decreases there is a decrease in titratable acidity and hydrogen ion concentration. The extent of the decrease in acidity depends on the time taken in filtration, the effect being connected with adsorption on the filtering material. The following table is given by Mehlitz.

TABLE 65.—FRACTIONAL MEMBRANE FILTRATION OF PECTIN SOLUTION.

Pore-size of Filter in μ.	Density.	Ca pectate in 100 gr. solution (gr.)	Acidity of 5 c.c. Filtrate in c.cs. N/100 NaOH.	pH at 18° C.
Unfiltered.	1·0039	0·348	14·0	3·00
> 3	1·0032	0·340	11·8	3·10
3	1·0030	0·332	11·0	3·10
2	1·0030	0·318	9·1	3·15
1	1·0028	0·250	8·2	3·15
0·75	1·0028	0·238	7·5	3·25
0·6	1·0027	0·230	7·4	3·30
ca 0·2	1·0012	0·038	6·9	3·40

[1] *Kolloid-Z.*, **41**, 130 (1927) ; cf. Sucharipa, *J. Assoc. Official Agr. Chem.*, **7**, 57 (1923).

[2] *Chem. Zeit.*, **30**, 921 (1906) ; *Z. Elektrochem.*, **12**, 777 (1906) ; **13**, 527 (1907) ; *Kolloid-Z.*, **1**, 107 (1906) ; **2**, 3, 33 (1907); *Z. physik. Chem.*, **60**, 257 (1906) ; *Biochem. Z.*, **6**, 379 (1910).

The viscosity of the sol decreases as the membrane pores decrease in diameter, but there is not a direct parallelism between the pectin content of the filtrate and its viscosity. Fig. 46 is an example of the type of result obtained by Mehlitz.

Owing to the non-pectin constituents of the apple-juice, viscosity cannot be directly correlated with the gelatinising power of the pectin solution. Mehlitz's results lead him to the conclusion that the specific viscosity of dissolved pectin corresponds with the degree of methoxylation and also that the degree of methoxylation is definitely related to the degree of dispersion of the particles.

Preliminary investigation revealed very little change in the surface tension of the filtrate through different pores, the tendency being a slight decrease in surface tension as the pore-size diminished.

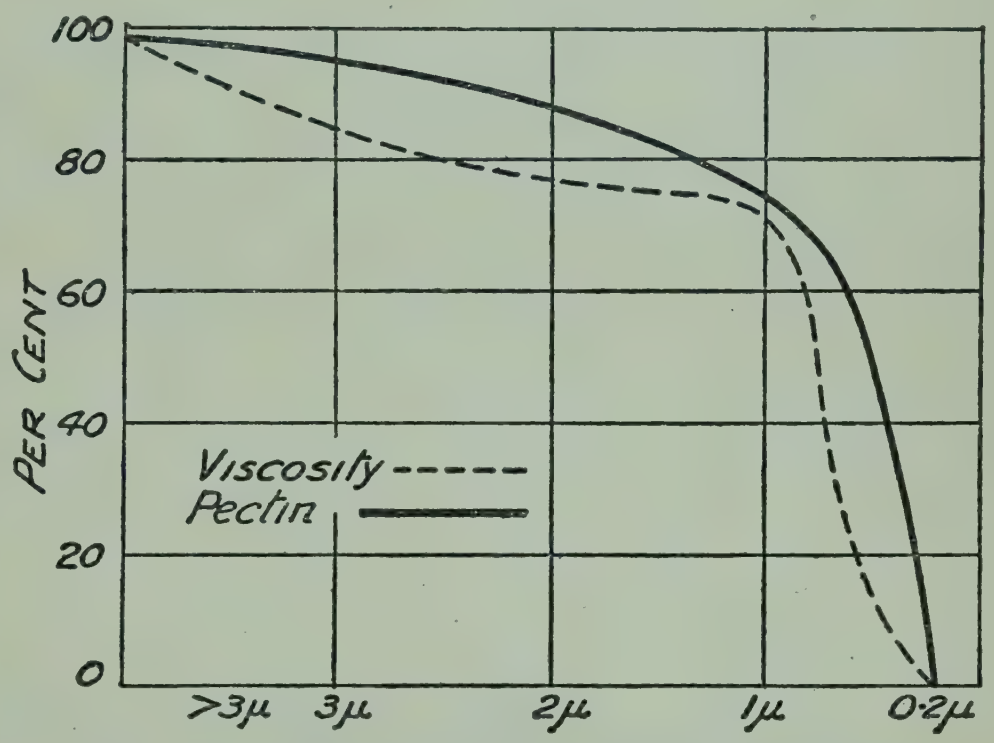


FIG. 46.—The relation between decrease in pectin and decrease in viscosity consequent upon fractional membrane filtration. Ordinates refer to (*a*) pectin content, (*b*) viscosity, the original values of both being taken as 100 per cent. Abscissæ are pore sizes of the filter.

The filtered fractions gave the reaction for starch, this being progressively feebler as the pore-size decreased. Even the filtrate passing about 0·2 μ still gave the starch reaction.

The various fractions were all capable of forming gels, except when the pore-size reached 0·2 μ; in this case a syrupy, thread-like structure developed. An almost complete parallelism was observed between the jellying power of the fractional filtrates and their methoxyl contents. The pectin constituents of the filtered fractions $>3\,\mu$ to $<0{\cdot}5\,\mu$ have an approximately constant methoxyl content of about 9 per cent. The pectin of the filtrate in the region 0·2 μ had a methoxyl content of only 2·94 per cent.

A pectin sol acts as a buffer to acids. Tarr [1] investigated the change in pH value when known amounts of tenth-normal acids were added to distilled water and 1 per cent. pectin solutions respectively. The presence of pectin materially reduced the dissociation of the acids; possibly actual pectin-acid compounds are formed. It was found that the magnitude of the pH change depends on the acid employed; Thus "to produce a hydrogen ion concentration of pH 3·50 with the pectin dissolved in the water requires approximately 2·6 c.c. of

[1] Univ. Delaware Agr. Exp. Sta., *Bull.*, **134** (Tech. No. 2), 20 (1923).

0·1 N. sulphuric acid, as compared with 5·5 c.c. of 0·1 N. tartaric acid, 7·5 c.c. of 0·1 N. phosphoric acid, or 8·5 c.c. of 0·1 N. citric acid."

Tarr believed that "there is a purely stoichiometrical relation existing between the combining power of the acids and the effect that they produce on the hydrogen ion concentration with pectin present in the solution. It is a relation that is based on the actual combining ability of the various acids." These conclusions, however, have been critically examined by Miss Spencer [1] who points out that pectin does *not* possess a buffer action tending to suppress the ionisation of acids. Tarr failed to take into account the presence of salt impurities, as well as a consideration of the actual pH value, instead of the change in pH value, when a definite acid increment was made. Miss Spencer finds no ground for the hypothesis of a pectin-acid compound, and no stoichiometric relation between pectin and acids in sugar-free solution. Again, the pH of an acid solution is not raised by pectin, which, however, by preferential adsorption of anions lowers the pH of mixtures.

PECTIN JELLIES

A mass of experimental data has been published on the problem of pectin jellies, involving the system : pectin, acid, sugar, water. It has long been recognised that satisfactory jellies only result when the pectin, sugar and acid contents fall within certain defined limits. The earlier work of Goldthwaite,[2] Cruess and McNair,[3] and Campbell [4] gave quantitative limits, but the more modern work has served to express the data, particularly regarding the pH value of the system, on a really scientific basis.

In 1922 Lal Singh [5] made a special study of the effect of the acid concentration. Using a citrus pectin and citric acid the following results were obtained, the pectin concentration being 1·5 per cent.

Relation of Acidity and Minimum Sugar Concentration to Jellying Capacity.

Total Citric Acid in Jelly.	Minimum Sugar Required per 100 gr. Final Jelly.
0·05 per cent.	75 grs.
0·17 ,,	64 ,,
0·3 ,,	61·5 ,,
0·55 ,,	56·5 ,,
1·05 ,,	53·5 ,,
1·55 ,,	52 ,,
2·05 ,,	50·5 ,,
3·05 ,,	50·0 ,,
4·05 ,,	50·0 ,,

[1] *J. Phys. Chem.*, **34**, 410 (1930).
[2] *Ind. Eng. Chem.*, **1**, 333 (1909) ; **2**, 457 (1910).
[3] *Ibid.*, **8**, 417 (1916).
[4] *Ibid.*, **12**, 558 (1920).
[5] *Ibid.*, **14**, 710 (1922). *Vide* Wendelmuth, *Kolloidchem. Beihefte*, **19**, 115 (1924).

The practical lesson from these results is that the commercial jelly maker should increase the acidity of his fruit juice and so economise on sugar. An increase in acidity from 0·1 to 1·7 per cent. resulted in a saving of nearly 20 per cent. of sugar.

The influence of the concentration of pectin was also investigated by Lal Singh. Maintaining the citric acid content constant at 1·5 per cent., and producing final jellies always to 100 gr. weight, the following relationships were found :—

Relation of Pectin Concentration to Jellying Capacity.

Pectin in Jelly.	Minimal Sugar in Set Jelly.
0·5 per cent.	No jelly possible.
0·75 ,,	,, ,,
0·9 ,,	65 per cent.
1·0 ,,	62 ,,
1·25 ,,	54 ,,
1·5 ,,	52 ,,
1·75 ,,	51 ,,
2·0 ,,	49·5 ,,
2·75 ,,	48 ,,
4·2 ,,	45 ,,
5·5 ,,	43 ,,

" Within certain limits, the higher the percentage of pectin in the juice the lower the amount of sugar required to form jelly. This fact is most noticeable in jellies containing 0·5—1·5 per cent. pectin." The commercial implication is to adjust the pectin and conserve sugar.

Goldthwaite [1] pointed out that the nature of the fruit acid influenced the jellying capacity of the system, tartaric acid being superior to citric acid. The observation was followed up by the comprehensive investigation of Tarr [2] in his study of " Fruit Jellies. I. The *Rôle* of Acids." This author places tartaric acid as the most efficient acid commonly present in fruit juices used for jellies. Citric acid is the least efficient, whilst malic acid stands intermediate.

Tarr found that there was no direct relation between *total* acidity and jelly formation. " If the activity of the acid is considered, however, the situation is quite the reverse. We find that while the total amount of acid that is necessary for jelly formation may vary over a wide range, the hydrogen ion concentration is practically constant for a particular juice and varies only slightly for different juices. With dried pectin, our purest source of pectin, this definite hydrogen ion concentration is pH 3·46."

[1] *Loc. cit.*
[2] *Loc. cit.*

The acids employed in the investigation were sulphuric, phosphoric, tartaric, citric and acetic. The anions had no noticeable influence. The amounts of the acids necessary for jellying were just those necessary to attain the critical pH value (the presence of salts, however, influences the situation, and this point is discussed later). The following results by Tarr are of interest in this connection :—

The Effect of Various Acids on 1 gr. of Pectin in 100 c.c. of Distilled Water.

Acid.	0·1 N. Acid Required to Produce :—		
	pH 3·46.	pH 3·21.	pH 3·00.
Sulphuric	2·78 c.c.	4·00 c.c.	5·40 c.c.
Phosphoric	8·00	12·70	17·50
Tartaric	5·80	11·12	18·60
Citric	10·00	19·80	35·60

The jellies were prepared using solutions containing 1 gr. of apple pectin and 62·5 gr. of sucrose, the sugar content of the finished

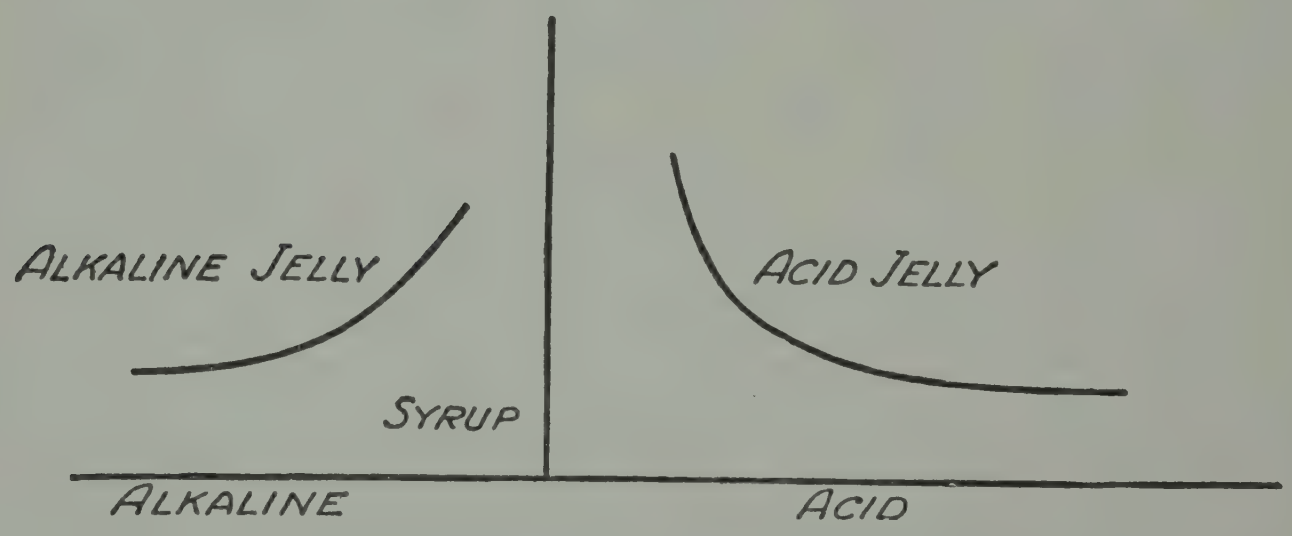


FIG. 47.—The acid and alkali jelly fields with constant pectin; ordinates are sugar content.

jelly being about 67·6 per cent. Not only is the formation of jelly controlled by the pH value of the system, but the *character* of the jelly itself depends on this too. In Tarr's experiments, a pH = 3·46 gave rise to a tender delicate jelly, which increased in stability until pH = 3·1—3·2 is reached. Below this pH value syneresis occurs and the jelly exudes liquid on standing. If the system is rendered still further acid, a white precipitate resembling pectin is thrown out. Excess acid inhibits entirely the jellying of pectin.

Little work has been reported concerning the effect of alkali [1] on pectin-sugar jellies. Miss Spencer [2] has presented a *qualitative* view of the jelly field and obtained jellies both in the alkaline and acid regions (see Fig. 47).

[1] Cf. Haynes, *Biochem. J.*, **8**, 553 (1914).
[2] *Am. Coll. Symp. Monog.*, **4**, 302 (1926).

Investigating the acid region, the sugar concentration (pectin constant) was carried to crystallisation. "At no point was there the slightest tendency toward syrup formation, so crystallisation and not syrup formation fixes the upper boundary of the acid jelly field." It is argued that previous workers who found syrups to result at high acid concentrations had overlooked hydrolysis. This is supported by the well-known fact that boiling weakens the jellying capacity of pectin. If the acid concentration is sufficiently high, pectin, will decompose in the cold. "This instability of pectin and not a limiting acidity for gelation will fix the right hand boundary of the acid-jelly field." Syrups exist only below the syrup-jelly boundary.

Miss Spencer's work opened up a new line of investigation and subsequently she employed a cold method for preparing sugar-pectin jellies.[1] Such jellies were readily formed in an alkaline medium, as well as in an acid medium. Several reasons led to the conviction that the alkaline jellies represent the same type of formation as the acid jellies, notably the fact that the pectin recovered from an alkaline jelly could be used to prepare an acid jelly.

Thus, there is not, as was previously believed, one sugar-acid-jelly zone for pectin bounded on all sides by a sol boundary, but two jelly zones, bounded by a sol region on the one side : low acid and low sugar. Theoretically, crystallisation of sugar fixes the limits of jellying for high-sugar systems, whilst pectin decomposition fixes the limits for high-acid or -alkaline systems. In practice, mechanical difficulties of mixing in advance of jelly-formation, fix the limits of homogeneous pectin jellies.

Considering Fig. 47 Miss Spencer showed, by experiments with alcohol and glycerol systems, that the meaning of the converging lines is that acid is not a *sine qua non* requisite for pectin jellies, but is necessary when the pectin precipitant has low solubility, *e.g.*, sucrose.

Work on pectin jellies was continued by Tarr and Baker,[2] studying the *rôle* of sugar. Sugar is essential for preparing a pectin jelly of satisfactory physical properties and for flavour. The actual physico-chemical importance of sugar still remains to be worked out. Pectin and sugar alone do not lead to jellies, acids or certain organic salts being necessary additions,[3] whereas pectin and acid only can give good jellies.[4]

Tarr and Baker investigated the problem along two lines : (1) a study of the equilibrium in the jellying system : pectin-sugar-acid-water ; (2) the inversion of sugar when making fruit jellies.

[1] *J. Phys. Chem.*, **33,** 1987 (1929).

[2] Univ. Delaware Agr. Expt. Sta., *Bull.*, **136** (Tech. No. 3), 1—29 (1924). See the criticism by Miss Spencer, *J. Phys. Chem.*, **33,** 1995 (1929).

[3] Cf. von Fellenberg, *Biochem. Z.*, **85,** 118 (1918).

[4] Ohn, *Ind. Eng. Chem.*, **22,** 639 (1930).

The results obtained have a direct practical bearing in the making of jams, marmalades and fruit-juice jellies.

The required amounts of pectin and acid were placed in an agate pan and brought to the boil on an electric hot-plate. Sugar was added, and boiling continued until the solution showed a tendency to break in sheet form from a silver spoon—the practical jam-boiler's test. The jelly was now weighed, transferred to a jelly glass, covered, and allowed to stand 12 hours at room temperature. It was then removed from the glass for examination.

The following table, due to Tarr and Baker, shows the quantitative aspect of jellying, the pH values being varied by means of sulphuric acid. Although the initial sugar quantities varied from 100 gr. to 180 gr., the percentage of sugar in the final jelly is approximately constant, the slight increase correlating with greater acidity.

TABLE 66.

The Effect of Sugar in Fruit Jelly Formation.

Wt. Pectin.	Wt. Sugar.	pH.	Wt. Jelly.	Per Cent. Sugar in Finished Jelly.
grs.	grs.		grs.	
2	100	3·37	144·0	69·4
2	105	3·37	152·5	68·8
2	110	3·37	159·0	69·2
2	115	3·37	166·0	69·3
2	120	3·37	173·0	69·4
2	125	3·37	182·0	68·7
2	130	3·37	185·0	70·0
2	135	3·23	193·0	70·0
2	140	3·23	199·0	70·0
2	145	3·23	202·0	71·3
2	150	3·10	209·0	71·8
2	155	3·10	216·5	71·6
2	160	3·10	223·0	71·7
2	170	3·10	234·0	72·7
2	180	3·10	252·0	71·4

A further table by Tarr and Baker reveals the effect on jelly formation of variations in the sugar concentration. Throughout the seventeen tests the initial pectin weight was 2 gr. and the initial sugar weight 100 gr.

Inspection of the table reveals: (1) weak and tender jellies result when the sugar content is low, irrespective of the pH value; (2) increase in hydrogen-ion concentration assists jellying. In this connection the authors report that with specially low ash pectins, good jellies were found with only 58·8 per cent. sugar when the pH value was 3·10.

TABLE 67. RELATION OF SUGAR CONCENTRATION TO JELLY CHARACTER.

pH.	Wt. Jelly.	Per Cent. Sugar in Finished Jelly.	Physical Character.
	grs.		
3·37	145·0	69·0	Tender, good.
3·37	148·0	67·6	Very tender, good.
3·37	152·0	65·8	Very tender, good.
3·37	156·0	64·1	Very weak jelly.
3·37	160·0	62·6	Practically liquid.
3·37	164·0	61·0	Liquid.
3·37	168·0	59·5	Liquid.
3·23	141·0	70·9	Excellent.
3·23	148·0	67·6	Tender, good.
3·23	156·0	64·1	Tender, collapsed.
3·23	160·0	62·6	Very tender, collapsed.
3·10	139·5	71·7	Very firm.
3·10	152·0	65·8	Tender.
3·10	156·0	64·1	Tender, collapsed.
3·10	159·5	62·7	Jelly collapsed.
3·10	164·0	61·0	Jelly collapsed.
3·10	168·0	59·5	Jelly collapsed at once.

Tarr and Baker have drawn attention to an important fact, viz.: that in a satisfactory jelly, the sugar content corresponds approximately with that of a saturated aqueous solution of sucrose. The slightly higher sugar content in jellies of low pH values corresponds to the increased solubility of sugar due to inversion. Data is given showing that at 20° C. a saturated solution of sucrose contains 67·09 gr. sucrose, whereas "by inverting to obtain maximum solubility, as much as 73·77 per cent. of cane sugar could be made soluble." The authors obtained the following figures from the Bureau of Standards, Washington, D.C.

Maximum Solubility of Cane Sugar in Invert Sugar.

Temp. °C.	Gr. Cane Sugar per 100 gr. Solution.	Gr. Invert Sugar per 100 gr. Solution.	Gr. Water per 100 gr. Solution.
20	37·1	38·6	24·3
25	35·7	41·4	22·9
30	33·4	45·6	21·0

The question of the inversion of sugar will be reverted to later (p. 373) owing to its technical importance in jam-boiling.

Miss Spencer [1] has dealt with the interesting question of the *sugar-holding capacity* of pectin, *i.e.*, the capacity of an acid-pectin sol to receive sugar and produce a gel. In technical practice this refers to the maximum quantity of sugar which can be added to a minimum amount of fruit juice to produce the maximum out-turn of jelly of desirable set.

Experiments confirmed the opinion that the "optimum sugar" value is *not* characteristic of pectin, but of the usual method of hot evaporation (cf. jam boiling), and, employing the cold method, no indication was observed of an optimum sugar concentration independent of other constituents.

A series of jellies was made up on the cold method with constant pectin and sugar but varying acidity and it was found that the curves connecting jelly-strength and acidity showed no tendency to pass through a maximum.

Miss Spencer concludes that "the tendency towards a closed acid jelly field with its optimum sugar and acid concentrations in the centre, does not represent the colloid behaviour of pectin," being really characteristic of the hot evaporation technique. It has no place, therefore, in the theory of the formation of the pectin jelly and, indeed, even the cold method of preparing gels is far too specific an instance to become the sole basis for an hypothesis since the solubility of sugar imposes a limit.

Since fruit jellies naturally contain salts of organic acids, the influence of these salts cannot be neglected by the practical food chemist. Halliday and Bailey [2] have published interesting data concerning the effect of calcium chloride on acid-sugar-pectin gels. The pectin was of apple origin and citric acid was employed. All gels were made to a constant weight of 100gr. With 1 per cent. pectin and 1 per cent. citric acid in the system, 50 per cent. sugar represented the minimum requirement for a firm gel.

The replacement in turn of different ingredients by $CaCl_2$ so as to yield firm jellies showed that the concentration of pectin, acid and sugar for gelation can be lowered by adding $CaCl_2$. The results are collected as follows, the figures referring to per cent. concentrations :—

Pectin.	Acid.	Sugar.	$CaCl_2$.
1	1	50	*Nil.*
1	1	46	0·5
1	0·3	50	0·5
0·5	1	50	1·0

[1] *J. Phys. Chem.*, **33**, 1996 (1929).
[2] *Ind. Eng. Chem.*, **16**, 595 (1924).

When $CaCl_2$ was present in excess of the requirements given above, syneresis occurred, a result suggesting that the pH value of the system is lowered due to $CaCl_2$ reducing the buffer action of pectin. It is possible, of course, that a calcium pectate is formed which gelatinises superior to plain pectin.[1] The results bear on technical practice wherein fruit juices frequently gel with a lower pectin content than would occur in plain aqueous medium.

A comprehensive study of the influence of salts on fruit jellies has been made by Myers and Baker,[2] using a purified apple pectin and the following salts: sodium sulphate, sodium hydrogen tartrate, and disodium hydrogen citrate. In preparing the jellies, the pectin, added sugar, and percentage of sugar in the finished jelly were

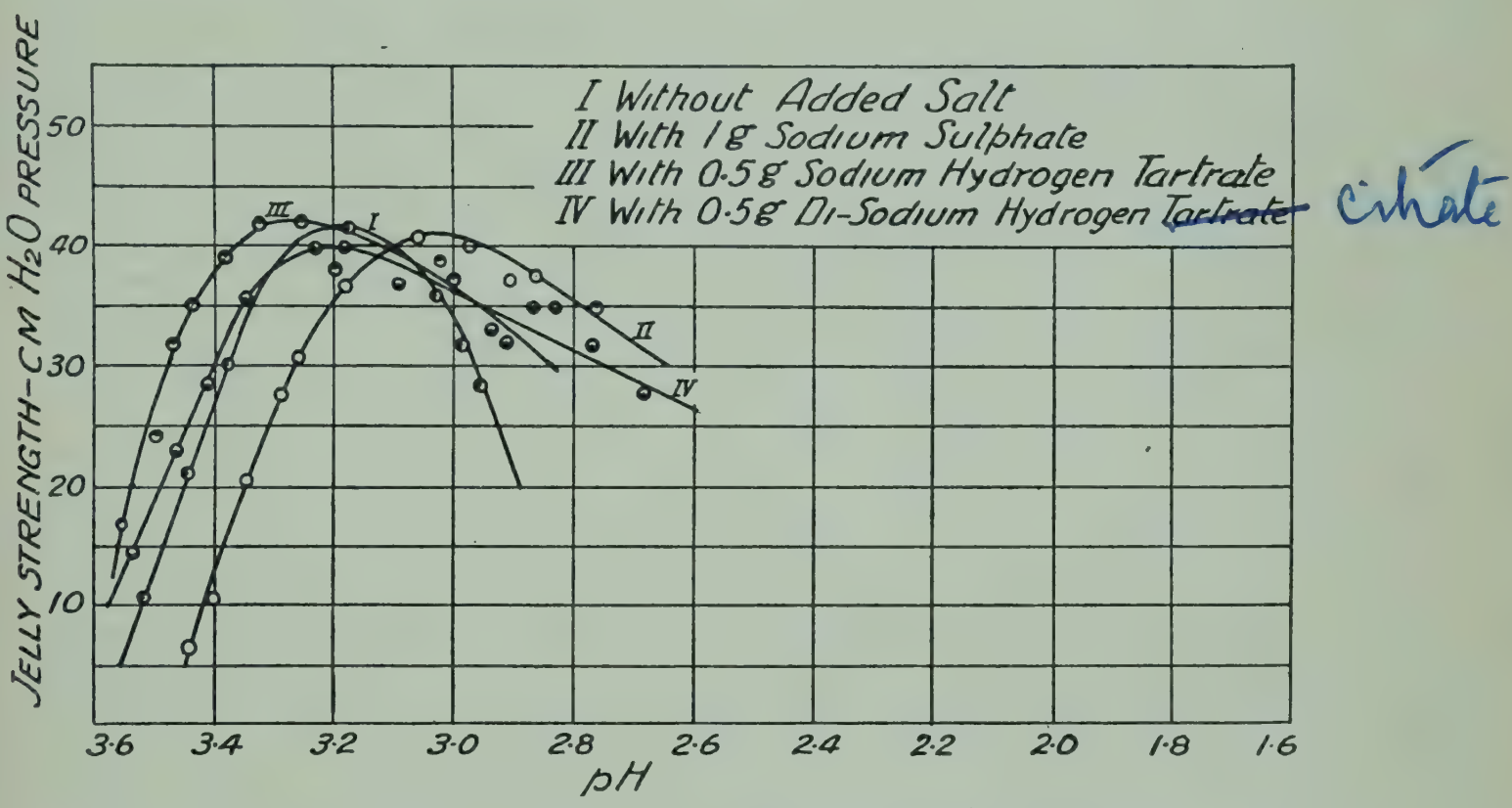


FIG. 48.—Curves showing the effect of hydrogen ion concentration on jelly formation, using tartaric acid with and without added salts.

maintained constant. Jelly strength measurements were determined using Tarr's apparatus.[3]

The graphical representation of the results obtained by Myers and Baker is illustrated in Fig. 48. It was observed that the curves for the different salts are in the same relative positions in respect to the curves for the acids only. The pH value for optimum jellying in the case of the acids alone is shifted, to the right by sodium sulphate and to the left by the tartrate and citrate. Fig. 49 records the buffer action of salts on the system acid: pectin; citric acid and citrate exert a marked buffer action. "Jellies made from highly buffered solutions are apparently less sensitive to changes in total acidity than jellies made from slightly buffered solutions." The

[1] De Waele, *Ann. physiol. et physicochim. biol.*, **5**, 864 (1929).
[2] Univ. Delaware Agr. Exp. Sta., *Bull.*, **144** (Tech. No. 7), 1—35 (1926).
[3] *Ibid.*, *Bull.*, **142**, (Tech. No. 5), 4 (1926).

curves connecting jelly-strength with pH value tend to flatten out when the more buffered solutions are employed. The practical significance of these results is that fruit juices contain salts which repress the ionisation of fruit acids, permitting good jellies to be obtained over a wider pH range. Syneresis is less liable than when working in the absence of salts, where the pH range is narrow and total acidity demands careful regulation. Pursuing this further, Myers and Baker showed that a salt could be added to a fruit juice of desirable pectin content, but of excessive acidity ; thus, it is possible to buffer such a juice to a pH value favourable to jelly formation.

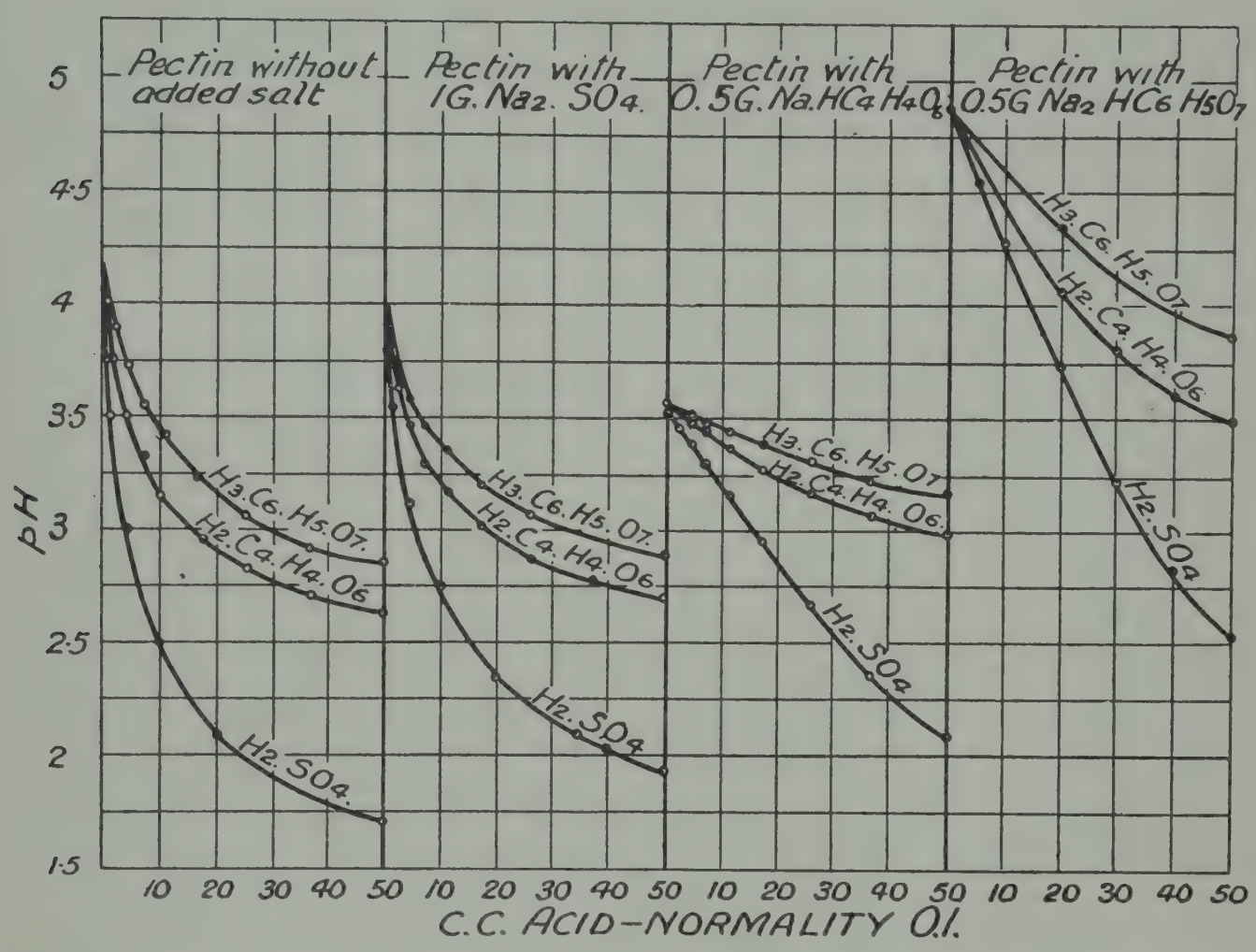


FIG. 49.—Electro titration curves of pectin and acid with and without added salts.

This salt effect is more pronounced in citric acid jellies than in sulphuric or tartaric acid jellies.

The investigation was now extended to determine the effect of the *cation* of the added salt on jelly formation. To this end, sugar, pectin and hydrogen ion concentration (using tartaric acid) were maintained constant, and the concentration of the salt (sodium hydrogen tartrate) varied. Fig. 50 shows the nature of the results obtained.

Myers and Baker draw certain conclusions from these graphs :—

pH 3.49. This pH value is nearly the minimum for jellying, but the tartrate raises the jellying strength to an optimum ; thereafter syneresis occurred and the jelly strength decreased.

pH 3.25. This pH value is close to the optimum for jelly formation. " The jelly strength decreases with increase in salt concentra-

tion up to a certain point and then increases considerably. This hydrogen ion concentration is very close to the hydrogen ion concentration, which will cause syneresis in the jelly, so that if the cation of the salt supplements the hydrogen ion in this capacity it should weaken the jelly and eventually cause syneresis. This is exactly what happened. Syneresis occurred at the points indicated on the lower part of the curve." On further addition of salt the syneresis ceased. Myers and Baker believe that as their

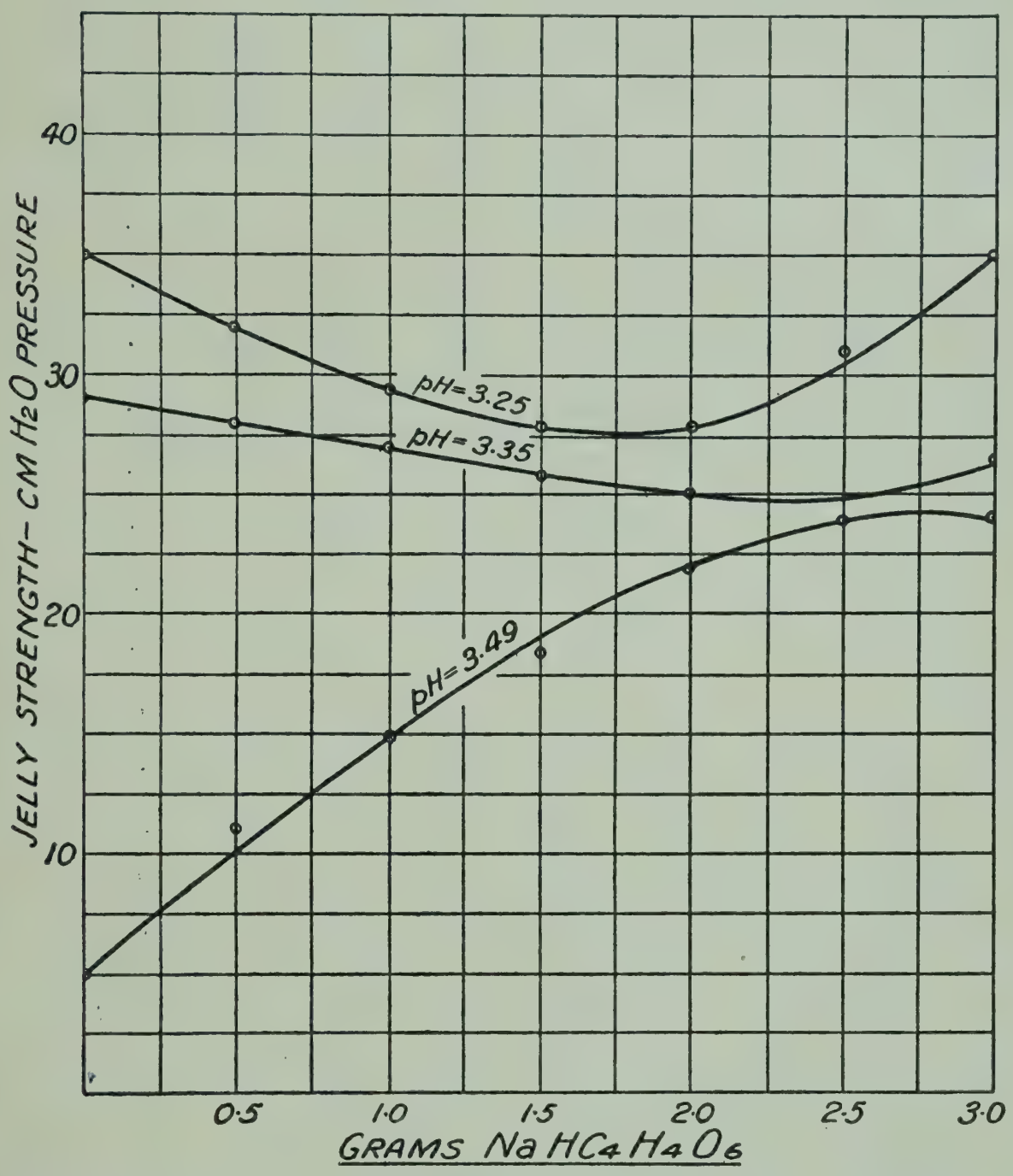


FIG. 50.—Curves showing the effect of a salt on jelly formation when the hydrogen ion concentration is maintained constant.

pectin had a negative charge, the addition of the salt caused the coagulation of the pectin by the cation. The pectin being coagulated, the jelly structure breaks down and liquid seeps out. As further salt is added, the protective effect of the anion comes into play, stabilising or peptising the pectin, and syneresis ceases. Such a phenomenon is familiar to students of colloid chemistry.

pH 3.35. The curve in this case lies intermediate between the other curves. The minimum point of the graph is at a salt concentration beyond that of the graph for pH 3·25, whilst the minimum

point on the graph for pH 3·49 would occur at an even greater concentration of the tartrate.

It was observed that cloudy, dull jellies, usual with sugar, pectin and acid systems became clear and sparkling when the salts were added, a fact of practical importance in connection with certain commercial marmalade jellies.

In a most interesting discussion, Miss Spencer [1] has dealt with the effect of salts on sugar-pectin jelly-formation and arrived at the conclusion that the stabilising anion charge on pectin can be neutralised by adsorbed cations, whether there be an organic precipitating agent present or not. In studying salt effects, the concentration of pectin and the technique of combining constituents are fundamentally important, as are also the acidity (or alkalinity) of the dispersing medium and the specific ions of the acid (or base) added.

The precipitation of pectin by salts occurs more readily in an alkaline medium. Miss Spencer postulates that a definite boundary line separates the sol and gel regions for this precipitation. Should there be intersection of the salt and acid (or alkali) concentrations within this gel region, precipitation will take place. Reversal of this precipitation can be effected by changing the salt-acid (or alkali) concentration relation to one lying in the sol region, *e.g.*, by lowering the pH value or by washing out the salt which favours precipitation.

The sugar precipitation of a given pectin hydrosol is enhanced by a given amount of a salt only if it lowers the sugar jelly boundary curve specific to this sol. Should it raise the boundary curve, jelly formation is inhibited or retarded.

Continuing their investigation of fruit jellies, Myers and Baker [2] have discussed in detail the *rôle* of pectin itself. The fundamental fact was established that the viscosity of a pectin solution affords an accurate measure of its jellying capacity,[3] and the viscosity of the pectin solution determines the quantity of sugar that the pectin solution will gel. A citrus pectin was used in 0·5 per cent. solution in distilled water, and the relation studied between the viscosity and the pH value.

The graph of the results showed that the viscosity of a pectin solution is at a maximum when in simple aqueous solution, addition of acid or alkali lowering the viscosity. The authors found this to hold true for several pectins, *e.g.*, an apple pectin having a pH value of 4·12 had its maximum viscosity at this (initial) pH value. The

[1] *J. Phys. Chem.*, **33**, 2012 (1929).

[2] Univ. Delaware Agr. Exp. Sta., *Bull.*, **149** (Tech. No. 8); 1—46 (1927).

[3] Later experiments by the authors (*loc. cit.*, *Bull.*, **160**, 40, 48 (1929)) showed that this is only true if the pectins are extracted under the same conditions, particularly at the same pH value. If the extraction is carried out at a pH value below 2·4, the jelly strength is not directly correlated with the viscosity.

decrease in viscosity on changing the pH value of the solution is ascribed to the increased dispersion of pectin by the acid and salts liberated. Increasing the hydrogen ion concentration only decreases the viscosity slightly.

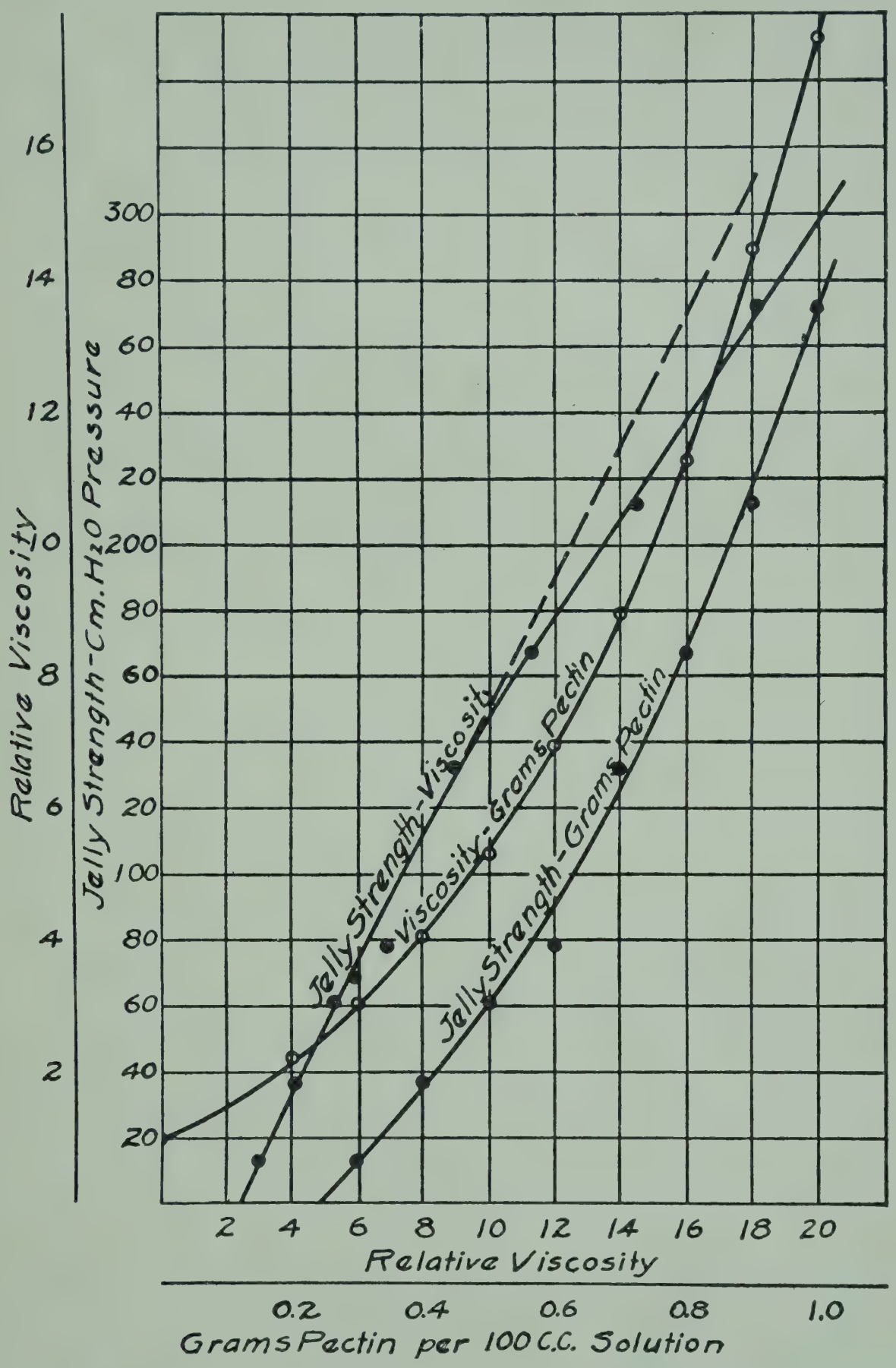


FIG. 51.—Curves showing the relationship between the viscosity of a pectin solution, concentration of pectin and the strength of the resulting jelly.

The relation between the viscosity of the pectin solutions at 26° C. and the strength of the jellies made by boiling with sugar to a final sugar percentage of 69·4 in the jellies is given in the following table, due to Myers and Baker, the jellies having stood at room temperature for 23 hours before measurements were made.

TABLE 68. VISCOSITY AND JELLY STRENGTH OF PECTIN JELLIES.

Pectin per 100 c.c. Solution (Grs.).	pH.	Relative Viscosity, 26° C.	Jelly Strength (Cm. Water Pressure).
0·2	3·16	2·22	No jelly.
0·3	3·11	3·02	13·0
0·4	—	4·05	37·5
0·5	3·00	5·30	59·0
0·6	—	6·97	77·0
0·7	—	8·97	132·0
0·8	2·88	11·26	166·5
0·9	—	14·43	210·0
1·0	2·76	18·13	275·0

The results are set out graphically in Fig. 51.

Obviously, jelly strength increases rapidly as the pectin concentration increases and is a direct function of the viscosity of the

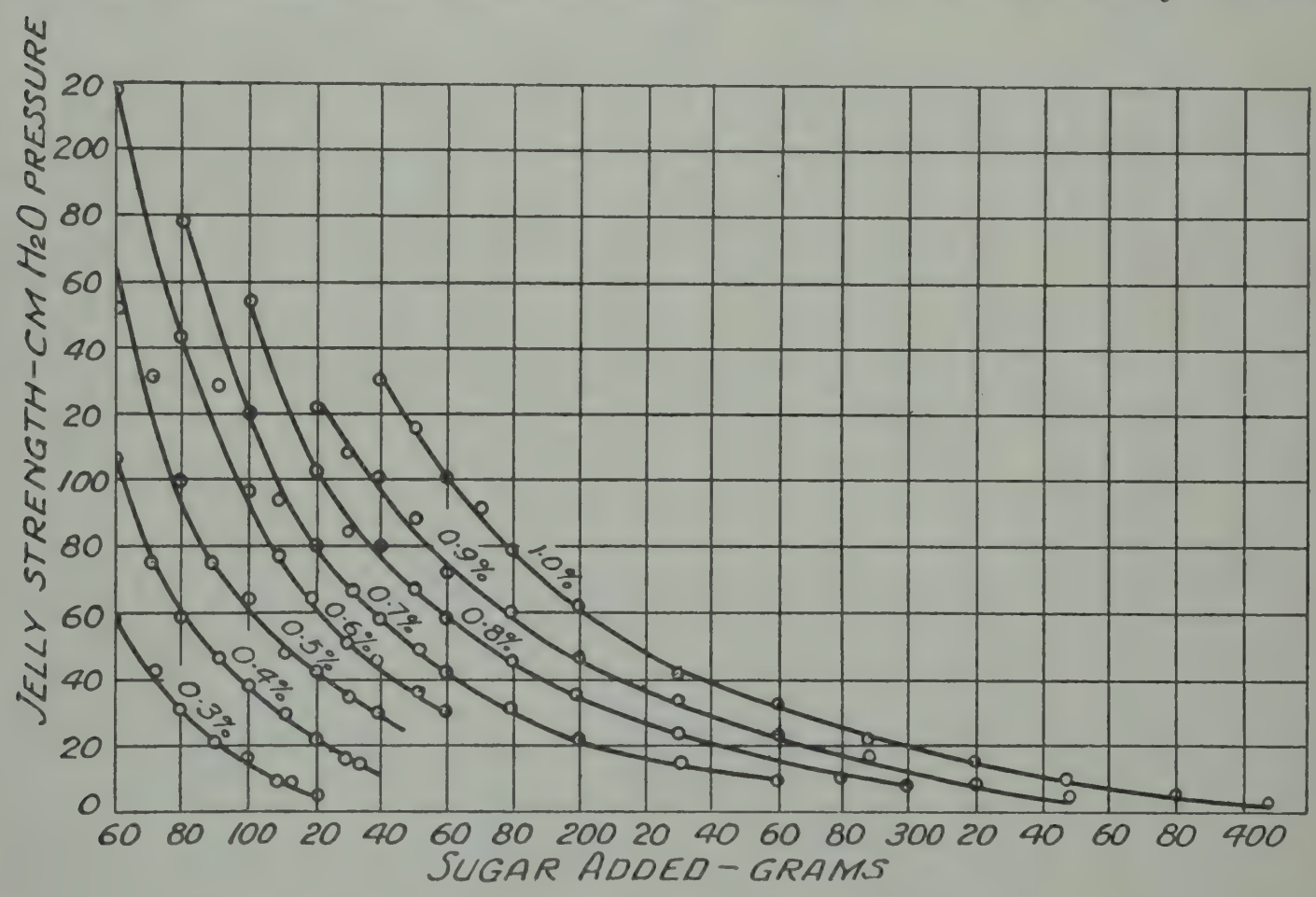


FIG. 52.—Curves showing the effect of added sugar on jelly strength.

pectin solution. No acid was added to the system. The decrease in pH value recorded here followed increasing pectin content, an unexpected result which merits further inquiry. Other experiments indicated that the jelly strengths are low throughout and a definitely linear relation would result if all the jellies had been tested at their optimum pH values.

Fig. 52 shows the effect of added sugar on jelly strength, working with pectin concentrations varying from 0·3 to 1·0 per cent., the viscosities varying from 3·02—18·13. Sugar was added to 100 c.c.

lots of pectin solution. The more viscous the pectin solution, the greater is the amount of sugar necessary to maintain constant the strength of the resulting jelly ; in fact, a linear relation holds good.

Ohn [1] has also made a detailed study of the viscosity of pectin sols, using a pure orange pectin, and agrees that measurements of the relative viscosity may be used as an indication of jellying power. Within certain limits the surface tension decreases as the relative viscosity of the sol increases, depending apparently upon the lowering of the pH value.

Later,[2] he studied the specific effect of citric acid, sucrose, fructose, dextrose and lactose on the viscosity and gelation of pectin sols *prepared and maintained at* 20° C. The presence of citric acid decreased the viscosity of pectin sols. Table 69 illustrates this and refers to the addition of citric acid crystals to 100 c.c. of pectin sol, the viscosity ratio being the ratio of the time of outflow of the sol at 20° C. in an Ostwald viscometer, and that of water.

TABLE 69. EFFECT OF CITRIC ACID ON THE VISCOSITY OF PECTIN SOLS.

Citric Acid (gr.).	0·1 per cent. Pectin.		0·4 per cent. Pectin.	
	pH.	Viscosity Ratio.	pH.	Viscosity Ratio.
0·0	5·69	1·82	3·77	4·59
0·1	2·97	1·70	—	—
0·2	—	—	2·66	3·99
0·5	2·67	1·62	2·57	3·73
1·0	2·50	1·58	2·33	3·71

Even in the presence of sugars addition of citric acid to cold-prepared sols leads to diminished viscosity.

As regards the influence of sugars under comparable conditions, sucrose leads to a higher viscosity than does dextrose, fructose or lactose, and in the pectin-sugar-acid system sucrose leads to firmer, more stable gels. The formation of sugar crystals in the gels occurred at a higher pH value in sucrose systems than in dextrose or lactose systems, whilst, under comparable conditions, fructose gels gave no evidence of sugar crystallisation.

An extensive investigation of the jellying of fruit juices has been carried out by Wendelmuth [3] whose results in general support the conclusions already arrived at above. Viscosity measurements are a feature of the work. Jellies were obtained without heating by intensive agitation of sugar with fruit juices. It is considered that

[1] *Ind. Eng. Chem.*, **18**, 1295 (1926).
[2] *Ibid.*, **22**, 635 (1930).
[3] *Kolloidchem. Beihefte*, **19**, 115 (1924).

the three-phase system, pectin-water-sugar, transforms into the two-phase system, hydrated pectin-hydrated sugar, of an emulsoid character. The pectin micelles form the framework of the jelly. Ageing weakens the jellies, probably because of the gradual splitting of the pectic acid ester into free acid and methyl alcohol.

Sucharipa [1] has also prepared jellies in the cold. Long heating and high temperature and pressure destroy jellying power of pectins.

NATURE OF THE PECTIN GEL

The nature of the pectin gel has been discussed by Miss Spencer,[2] who shows that gelation in this instance is entirely a phenomenon of precipitation, not swelling. Pectin is negatively charged in alkaline and acid media, thus precluding isoelectric precipitation, and it adsorbs water. Hence, the sol is stabilised by water and by adsorbed anions. Addition of organic reagents, such as sugar, affects the pectin-water equilibrium, destabilising the pectin and leading to its conglomeration. In this connection is mentioned the fact that dry pectin cannot be satisfactorily peptised in a dispersing medium, wherein the sugar concentration is greater than about 30 per cent. In other words, this is the minimum amount of sugar which will permit gel formation with a 1 per cent. pectin hydrosol. Again, since increasing acidity or alkalinity decreases the sugar (*i.e.*, organic precipitant) necessary for the gelation of pectin, the maximum amount of water adsorbed by pectin will be in the region of pH 7·0.

The conditions which bring about the formation of pectin gel are those which lead to a network of conglomerates able to support the weight of the mother liquor, the strength of such a structure being a function of its continuity and its rigidity. The number and proximity of precipitated pectin particles, depending on the concentration and degree of dispersion of the pectin, will determine the continuity factor, whilst the rigidity factor is controlled by the precipitating tendency of the dispersing medium. This will vary with the sugar concentration and the pH value obtaining.

Experiments supported the views that a dense and water-rich gel is given by high pectin and low sugar-acid ; a dense and rigid gel is given by low pectin and high sugar-acid. Variations in rigidity depend on the amount of water held by the pectin when precipitated and are determined, therefore, by the concentration of sugar (for constant acidity) or the concentration of acid (for constant sugar).

Ohn [3] has put forward interesting speculations regarding the effect of acids on the fibrils of pectin. Acid distends and toughens the fibrils permitting them to support sugar syrup in the inter-fibrillar spaces. With too little acid, a weak fibrillar structure

[1] *J. Assoc. Official Agr. Chem.*, **7**, 57 (1923).
[2] *J. Phys. Chem.*, **33**, 2001 (1929).
[3] *Ind. Eng. Chem.*, **22**, 638 (1930).

cannot support sugar syrup and a weak gel is the result. When the acid is too high, destruction of the elasticity of the fibrils occurs and tough gels result. Further work is promised, using ash-free pectin, similar to that of Sponsler and Dore [1] on the structure of ramie cellulose.

JELLY STRENGTH

Many experiments have been carried out to determine quantitatively the optimum jelly conditions of pectin jellies by measuring the jelly strength. Different workers employ different technique, but the results accord quite well.

Lüers and Lochmüller [2] measured the resistance of the jelly to tearing apart, the jelly being set about a tearing tool in a special apparatus, the arrangement being such that on applying a known force the tool is pulled upward through the jelly (Lüer's pektinometer).

For constant sugar concentration, gelation is directly proportional to pectin concentration. The effect of variation in pH value is very marked, the strongest jellies being at pH 2·95 to 3·05. The gel strength falls slowly on decreasing the pH value from the optimum, but very rapidly on increasing it. Beyond pH = 3·45 no jellies resulted. For constant pectin and increasing sugar, jelly strength increases. Boiling the mixture reduces gel strength because of hydrolysis. A close proportionality exists between jellying capacity and the methoxyl content of the pectin. Pectins of less than 7·3 per cent. methoxyl did not gel, whilst pectins with 11—12 per cent. methoxyl gave the best jellies. The following table by the authors illustrates the results obtained :—

TABLE 70.

Test.	Methoxyl Content.	Tearing-Force.	$\frac{\text{Tearing-Force}}{\text{Methoxyl}-7\cdot3}$
	per cent.	grs.	
1	7·3	—	—
2	7·9	54	90
3	8·2	104	115
4	8·3	132	132
5	8·7	145	104
6	9·4	220	105
7	9·8	290	113
8	10·2	320	110
9	10·9	300	83
10	11·2	400	103
11	11·4	380	93
12	11·7	440	100
			Mean 105

[1] *Am. Coll. Symp. Monog.*, **4**, 174 (1926).
[2] *Kolloid-Z.*, **42**, 154 (1927).

These results, however, are not confirmed by the work of Myers and Baker,[1] who found that "there seems to be no correlation between the methoxyl content and jelly grade of the pectins. The pectins of highest methoxyl content were not the highest in jellying power." These authors are now investigating the constitution of pectin in order to throw light on these discrepancies.

Fellers and Griffiths [2] testing fruit jellies with the Bloom gelometer [3] found that weak jellies set more slowly than firm ones, the

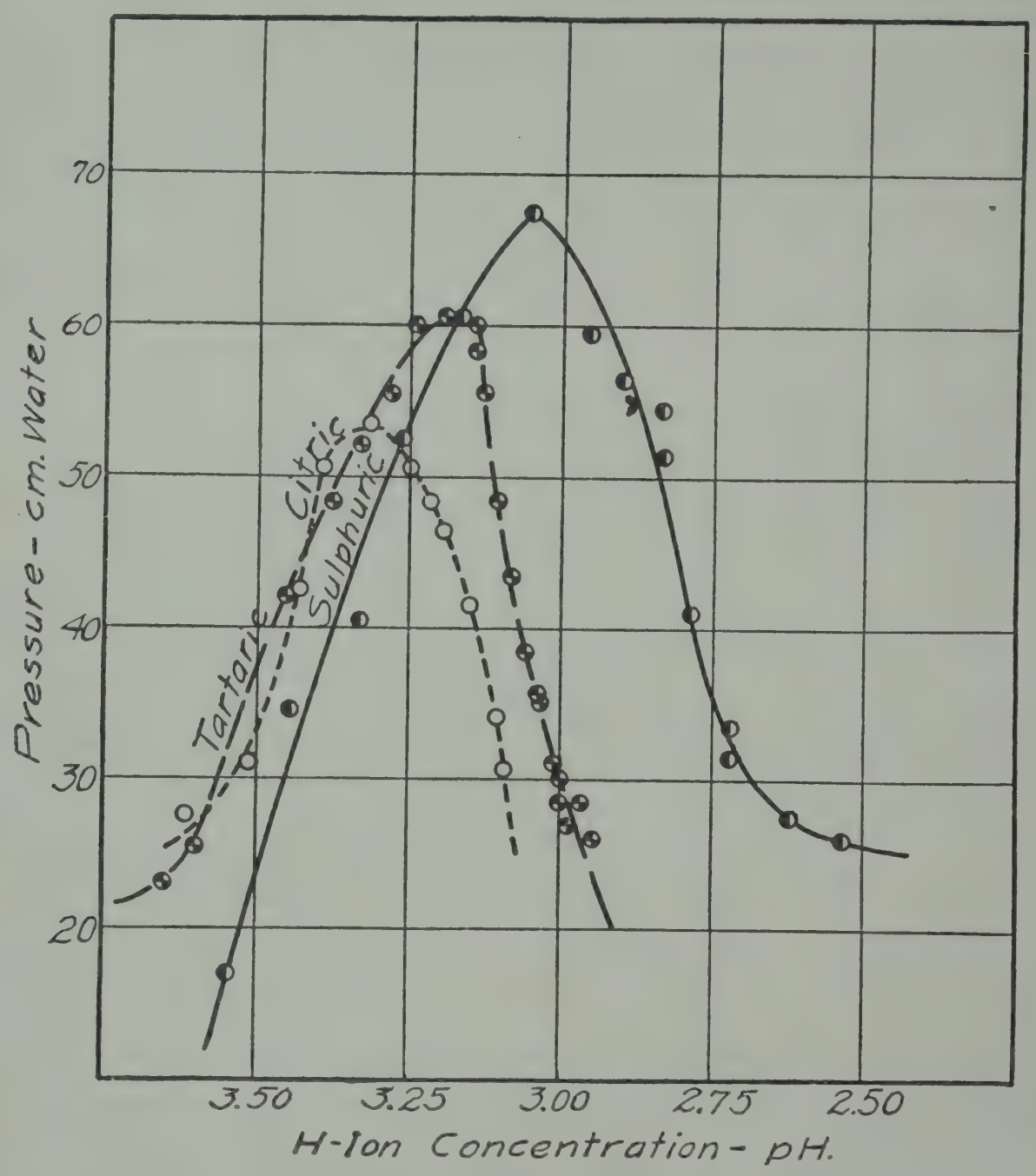


FIG. 53.—Combination curve showing the effect of hydrogen ion concentration on jelly strength.

greater part of the change occurring during the first 24 hours, the gradual increase lasting over several days. Although Tarr finds the opposite, the authors observed that the surface of an uncovered jelly was markedly toughened on storage. In the case of fruit juice jellies the bottoms of the samples were less strong than those at the top, the reverse effect to that obtaining with artificial pectin jellies.

Baker [4] investigated jelly strength by forcing a syringe plunger

[1] Univ. Delaware Agr. Exp. Sta., *Bull.*, **160** (Tech. No. 10), (1929).
[2] *Ind. Eng. Chem.*, **20**, 857 (1928).
[3] Cf. Richardson, *Chem. Met. Eng.*, **28**, 551 (1923).
[4] *Ind. Eng. Chem.*, **18**, 89 (1926).

into the jelly, pressure being developed on the plunger by the displacement of air by water in a bottle and recorded on a manometer. The apparatus and method and also the results obtained are described in detail by his colleague Tarr.[1]

In studying the effect of acids all jellies were made from 1 gr. pectin, added acid, and water to make 120 c.c. total volume. The mixture was brought to the boil and 100 gr. sucrose added. Boiling was continued until the system weighed 144 gr., *i.e.*, sugar content = 69·4 per cent. The jelly was cooled and tested 18 hours later. Fig. 53 shows the relation between hydrogen ion concentration and jelly strength.

The pH value for optimum gelation varied with the acid employed, being least with citric acid. The data support previous work of the Delaware laboratory "to the effect that a pectin-acid compound is formed, or that some compound is formed with the acid and a product split off from the pectin At the hydrogen ion

TABLE 71. THE EFFECT ON JELLY STRENGTH OF VARYING THE CONCENTRATION OF SUGAR IN THE FINISHED JELLY.

Wt. Finished Jelly (gr.)	Per cent. Sugar in Jelly.	Jelly Strength Pressure cm. Water.	
		Top.	Bottom.
135·0	74·1	63·5	57·5
135·0	—	64·0	55·5
138·0	72·5	65·5	67·0
140·0	71·4	62·5	68·5
140·5	71·0	67·5	72·5
140·5	—	66·0	73·0
144·0	69·4	63·5	86·5
144·0	—	63·5	90·0
144·0	—	64·5	86·5
145·0	69·0	63·5	89·5
145·0	—	63·5	89·5
146·0	68·5	58·5	83·5
147·0	68·0	58·5	81·0
148·0	67·6	55·5	82·5
148·0	—	55·5	83·5
148·5	67·3	52·5	74·5
150·0	66·7	44·5	63·5
150·5	66·4	44·0	62·0
152·0	65·8	41·5	58·5
155·0	64·5	34·0	36·0
157·0	63·7	32·0	28·5
160·0	62·5	28·5	19·0
162·0	61·7	25·5	16·0
165·0	60·6	17·5	4·0

[1] Univ. Delaware Agr. Exp. Sta., *Bull.*, **142**, (Tech. No. 5), 1—33 (1926).

concentration existing when fruit jelly formation occurs—that is, below pH 4·0—weak dibasic and tribasic acids, such as tartaric and citric acids, would have only one hydrogen ion free for combining purposes. The action of sulphuric acid would be similar to that of a strong monobasic acid. If a combining reaction is occurring, therefore, the relation of sulphuric acid : tartaric acid : citric acid would be as 1 : 2 : 3 " The data obtained supports this relation. Assuming pectin-acid compounds to form, the effect on pH value would be most pronounced with citric acid and least with sulphuric acid " The significance of the optimum jelly forming at different hydrogen ion concentrations, depending entirely on the particular acid involved, thereby becomes readily apparent."

The effect of the concentration of sugar in the finished product on the jelly strength was determined, using jellies containing constant pectin and tartaric acid. Table 71 records the results obtained.

It is seen that jellies form in the presence of an approximately saturated solution of sugar.

COMMERCIAL APPLICATIONS

The foregoing data has important application in the technical production of fruit jellies, jams and marmalades. These are pectinous jellies containing pectin, sugar and fruit juices, jams containing also the fruit itself, whilst marmalades are similar preparations made from oranges and/or lemons. From a bacteriological standpoint the total sugar content should be about 67 per cent., of which from 30 to 40 per cent. is invert sugar. Sugar is necessary, too, in respect to flavour, texture and yield.

The present volume is not concerned with the minutiæ of the technical preparation, but certain points from the previous discussion will be considered.

The raw material is fruit. Some fruits make into jam easily, others require admixture of other fruits or fruit juices to permit of a satisfactory set. In other words, the pectin requirement needs supplementing by adding a small portion of a pectin-rich fruit. Sometimes the added fruit juice is for the purpose of supplying acid.

The hydrogen-ion concentrations of fruit juices vary according to the condition of the fruit, but the typical pH values for the more common fruits are shown in Table 72.

A comprehensive study of the nature of the acids present in fruits has been made by Bigelow and Dunbar,[1] whilst analytical data on the total titratable acidities are given by Plimmer,[2] Wiley [3]

[1] *Ind. Eng. Chem.*, **9**, 762 (1927).
[2] " Analyses and Energy Values of Foods " (London, 1921), 201—8.
[3] " Foods and Their Adulteration " (London, 1917), p. 369.

TABLE 72. pH VALUES OF FRUIT JUICES

Fruit.	pH.	Ref.
Apple	3·8	1
	3·2—3·5	2
	3·05	3
	4·6	1
Banana	4·6	1
	5·0	4
Blackcurrant	6·0	5
Cherry	2·5	1
Currant	3·3	2
Gooseberry (unripe)	4·4	5
,, (ripe)	4·8	5
Grape	4·5	1
Grape fruit	3·0	1
Lemon	2·2	1, 2
Lime	1·7	1
Orange	3·1	1
,,	4·1	2
Peach	3·6	2
Pear	4·2	1
,, (ripe)	4·5	6
,, (unripe)	2·07	6
Pineapple	3·4	1
Plum	3·3	2
Quince	3·2	2
Rhubarb	3·1	1
,,	4·8	5
Strawberry	3·4	1

and Brooks.[7] Citric acid predominates in cranberries, currants, pears, raspberries, and strawberries. Malic acid predominates in apples, cherries, peach, plums and quince.

The pectin and sugar contents of fruits vary widely, as published analytical data reflect. The following table collects some of the data given by Lampitt and Hughes,[8] Mansfield,[9] and Macara.[10] The total sugar is usually stated in terms of invert sugar and the acidity as citric acid (stone fruits are analysed after removal of the pits); all values are percentages.

[1] Morse, "Applied Biochemistry" (Philadelphia, 1927), p. 592.
[2] Wendelmuth, *Kolloidchem. Beihefte*, **19**, 115 (1924).
[3] Andrews, *Proc. Indiana Acad. Sci.*, **34**, 265 (1924).
[4] McGuire and Falk, *J. Gen. Physiol.*, **4**, 437 (1922).
[5] Ziegelmayer, *Kolloid-Z.*, **52**, 243 (1930).
[6] Cortese, *Ann. chim. applicata.*, **20**, 197 (1930).
[7] "Critical Studies in the Legal Chemistry of Foods" (New York, 1927), 16.
[8] *Analyst*, **53**, 32 (1928). *Vide* also Brooks, *loc. cit.*, p. 55.
[9] *Food Manfr.*, **1**, 14 (1927).
[10] *Analyst*, **56**, 35 (1931).

TABLE 73. ANALYSES OF FRUITS USED IN JAMS

Fruit.	Lampitt and Hughes.		Mansfield.			Macara.		
	Pectin (av.).	Total Sugars.	Pectin (av.).	Total Sugars.	Acidity.	Pectin (av.).	Total Sugars.	Acidity.
Apples . .	1·29	7·11	0·75	4·03	1·10	0·75	7·60	1·11
Apricots . .	1·03	5·19	—	—	—	—	—	—
Blackberries .	0·94	3·48	—	—	—	0·59	5·10	0·85
Blackcurrants .	1·52	5·50	0·86	4·95	2·34	1·08	6·44	3·48
Cherries . .	0·35	11·47	—	—	—	0·24	8·33	0·88
Damsons. .	—	—	1·62	6·66	2·01	1·15	7·53	2·48
Gooseberries .	1·08	3·98	—	—	—	0·81	3·51	2·22
Greengages .	1·14	6·45	1·07	5·10	1·37	0·95	8·00	1·20
Oranges (peeled)	—	—	1·20	3·50	4·06	—	—	—
Plums (Victoria)	0·96	6·31	1·10	4·28	1·51	0·81	7·43	1·64
Raspberries .	0·71	4·80	0·27	1·81	1·4	0·53	3·53	1·73
Red Currants .	1·17	5·38	0·47	5·65	2·26	0·58	4·80	2·54
Strawberries .	0·68	5·56	0·41	4·52	0·99	0·53	5·48	0·93

Most fruits contain pectin to permit of jam-boiling direct. Where necessary, apple juice or gooseberry juice serves to increase the pectin content, *e.g.* with strawberries and sometimes raspberries. The sugar and fruit are very rapidly boiled in open pans (although vacuum pan methods are coming into prominence) until the total sugar concentration is about 67 per cent. As soon as the jam-boiling is complete, the contents of the pan are emptied into a cooler. Cooling has to be carefully controlled so as to avoid air-bubbles and/or " floating " fruit.

Detailed analyses of jams are available by several authorities [1] and the average pectin contents are given by Brooks [2] and King.[3] The latter author finds the following pectin content in market jams, expressed as calcium pectate :—

Jam.	Calcium Pectate.
Blackberry and apple . . .	0·25 per cent.
Damson	0·35 ,,
Gooseberry	0·45 ,,
Plum	0·46 ,,
Plum and apple	0·13 ,,
Raspberry	0·31 ,,

Jam must possess a good " set " in order to transport readily, inhibit fermentation [4] and appeal to the consumer. To attain the

[1] Brooks, *loc. cit.*, p. 45 ; Wiley, *loc. cit.*, p. 377 ; Plimmer, *loc. cit.*, p. 212 ; McGill, *Lab. Inland Rev. Dept. Ottawa*, Canada, *Bull.*, **302**, 1—23 (1915) ; Beythien, *Z. Untersuch. Lebensm.*, **56**, 80—106 (1928) ; Shutt, *Analyst*, **55**, 392 (1930).

[2] *Loc. cit.*, p. 55.

[3] *Analyst*, **50**, 371 (1925).

[4] Hirst, *Food Manfr.*, **3**, 447 (1928).

desired condition of jelly, the pectin, sugar and acid contents must be carefully controlled. The acidity (as malic acid) varies from 0·4—1·4 per cent. depending on the fruit and juices used.

The modern laboratory control of jam-boiling aims at keeping the sugar at a scientific but economical level (65—67 per cent.), pectin adjusted by adding pectin solution or pectin-rich fruit juice, and pH value in the optimum jellying range of 2·9—3·2.[1] The pH value is fundamental in regard to a proper set ; too stiff a jelly is unpalatable and is subject to syneresis.

Accurate boiling is necessary to avoid (*a*) too great a total sugar content in the final product, (*b*) over-inversion of sugar, (*c*) hydrolysis of pectin.[2] Nanji and Norman [3] have proved the marked hydrolysis undergone by pectin on heating with tartaric acid at 100° C. Increase in the concentration of the acid above 1 per cent. greatly increases the degree of hydrolysis. This means that in technical practice added pectin should be employed as late as possible in the process. Vacuum boiling is of advantage in conserving the jellying-power of pectin. Jameson [4] suggests pasteurisation of the pectin solution and then cooling the boiling jam or jelly to pasteurising temperature before adding the pectin. Such a method would be advantageous in the case of large-bulk packages which retain their heat for several hours. A study of the *rate* of setting of pectin jellies is called for, as this aspect of the problem has not received the attention it merits.

The phenomenon of the " curdling " of jellies has been discussed by Miss Spencer.[5] Curdling is due to the premature precipitation of pectin when sugar is added to hot fruit juice rich in pectin and acid. On cooling to the requisite temperature, jelly commences to form before the sugar is all dissolved and the jelly is thus distributed heterogeneously as small centres where pectin and sugar crystals meet. On raising the temperature to boiling, there may not be a repeptisation of these jelly centres and being therefore unavailable for bulk jelly formation, they produce a system of impaired jelly strength. When there is an excessive number of such jelly centres, the phenomenon of curdling is observed.

Discussing the preparation of fruit jellies, the same worker points out that the position of the gel-sol boundary for any cooked jelly depends on : (*a*) the content and condition of pectin in the original fruit juice, (*b*) protective colloids and electrolytes in the juice, (*c*) thermal stability of the pectin. " The quantitative values

[1] Mehlitz, *Konserven-Ind.*, **12,** 467 (1925) ; *Z. tech. Biol.*, **11,** 134 (1925) ; Eckart and Gyalokay, *Konserven-Ind.*, **14,** 2, 13, 37 (1927) ; Leo, *The Canner*, **61** (2), 41 (1925).

[2] Cf. Mehlitz, *Chem. der Zelle Gewebe* **12,** 353 (1926) ; *Chimie et industrie*, **16,** 301 (1926) ; *Konserven-Ind.*, **13,** 1 (1926).

[3] *J. Soc. Chem. Ind.*, **45,** 340 T (1926). See also, Myers and Baker, Univ. Delaware Agr. Exp. Sta., *Bull.*, **160,** 23 (1929).

[4] *Ind. Eng. Chem.*, **17,** 1292 (1925).

[5] *J. Phys. Chem.*, **33,** 1993 (1929).

for the gel-sol boundary are specific for each fruit." For example, the cranberry jelly field is large, apple jelly smaller, and strawberry (low in pectin) and elderberry still smaller. The last named is probably due to salts or protective colloids which stabilise the pectin sol.

When the jelly field is large, the preparation of jelly is easy because of the more extensive range existent for acid and sugar. When it is small, jelly preparation is rendered difficult, since one can miss the right point and instead arrive at a system which is in the sol zone for the fruit in question.

Myers and Baker [1] have also studied the deleterious effect of heating pectin with acid. Their results open up interesting technical possibilities regarding fruit juice jellies, inasmuch as their data " check well enough to warrant the conclusion that jelly strength is a function of the viscosity regardless of concentration of pectin. The relationship between viscosity and added sugar (presented in their data) is therefore, also independent of concentration of pectin. Jelly making is therefore simplified to a considerable extent since it is only necessary to measure the viscosity of a pectin solution with an Ostwald viscometer at 26° C., adjust the solution to that hydrogen ion concentration corresponding to the optimum point of jelly formation, add that amount of sugar corresponding to the viscosity for each 100 c.c. of solution and boil until the jelly contains 69·4 per cent. sugar." The technical chemist would of course have to prepare his own data along these lines with the particular pectin being used and work to his chosen sugar maximum.

Fruit juices contain organic salts and other buffers which have to be taken into account, particularly in the case of fruit-juice jellies and clear marmalades. The brilliance and stability of the jellies are involved, as the work of Myers and Baker has shown.[2] At the same time no quantitative work seems to have been published *re* the relation of turbidity or opalescence of pectin jellies in relation to pH value and the presence of organic salts. Myers and Baker summarise their results thus: the buffer action of salts in fruit juices is more marked in the case of weaker acids than strong acids. The jelly strength is less sensitive to hydrogen ion concentration variations when the system is buffered. " The anion of the salt functions mainly as a buffering agent and may also function as a peptising agent if present in sufficient quantities, thereby increasing the strength of jellies and preventing syneresis. The cation of the salt functions in a manner similar to that of the hydrogen ion of the acid, but only at definite hydrogen ion concentrations. Syneresis in jellies may be caused by the hydrogen ion alone or by the hydrogen ion and cation of the salt together, but not by the action of the

[1] *Loc. cit.*, *Bull.* **149** (1927), p. 25 *seq.*
[2] *Loc. cit.*, *Bull.* **144** (1926).

cation of the salt alone." Other work by various authors [1] on pectin jellies is directly of interest in technical practice.

Marmalade offers special opportunity for the technical utilisation of pectin. According to Lal Singh [2] waste lemon peels yield about 4·6 per cent. pure powered pectin or over 90-lb. per ton. "The peels can be boiled separately and the extract or purified pectin added to orange juice to supply pectin, or it can simply be added to sliced oranges and boiled with the latter for extraction of juice to make jelly or marmalade." Marmalade prepared in this country from bitter varieties of oranges contains sufficient pectin and acid to produce a firm jelly, but American marmalade made from sweet oranges is frequently admixed with grape fruit to increase the pectin and acid content.[3]

The question of the inversion of sugar, already mentioned, has been raised by Fiedler [4] who stated that over-inversion of sugar in jam or marmalade boiling inhibits gelatinisation and may even prevent it entirely. However, his results can be interpreted on the besis of hydrolysis of pectin by acid and the fact remains that honey (69 per cent. invert sugar) can give good pectin jellies.[5] Again, good jellies may be prepared using glucose-syrup or sucrose inverted by means of invertase to 96 per cent. inversion. No difficulty is observed in such jellies in respect to gel strength, clarity or syneresis. Also, it has been shown that in sucrose-acid-pectin jellies (pH = 2·09) stored at 28° C. inversion of the sucrose is taking place, the percentage inverted being 28·8 after 1 day and 99·0 after 113 days.[6] The fact remains that invert sugar [7] is regularly employed in jam manufacture, the aim being to have a total sugar content of about 67 per cent., and the ratio of sucrose to invert sugar as 1 : 1·5. Such a ratio is the optimum from the standpoint of the microbiological stability of jams and marmalades.

One point of great importance to the technical chemist in his work on jellies, jams and marmalades has received singularly little attention. One refers to the "ageing" of pectinous jellies on storage. Ageing phenomena are common in colloid systems generally, but Wendelmuth [8] alone seems to have studied the ageing of pectin solutions but not of jellies. A valuable field of inquiry

[1] Papa-Ioannu, *Ann. Gembloux*, **32** (3), 49—61; (4) 81—92; (7) 145—59; (8) 179—93 (1926); Lindemann, *Meldinger Norges Landbruks*, **7**, 157 (1927); Paine, *Am. Food J.*, **17**, (3) 11—13 (1922); Barker, *J. Soc. Chem. Ind.*, **37**, 243 T (1928); Lippold, "Obstkonserven, Fruchtsäfte, Marmaladen" (Leipzig, 1916); Griebel and Northnagel, *Z. Untersuch. Lebensm.*, **49**, 352 (1925).

[2] *Ind. Eng. Chem.*, **14**, 711 (1922).

[3] Cruess, "Commercial Fruit and Vegetable Products" (New York, 1924), p. 280.

[4] *Konserven-Ind.*, **15**, 405, 427 (1928).

[5] Joseph, *Food Manfr.*, **5**, 257 (1930).

[6] Cole, Cox and Joseph, *ibid.*, **5**, 165 (1930).

[7] *Vide* Jordan, *Ind. Eng. Chem.*, **16**, 307 (1924).

[8] *Kolloidchem. Beihefte*, **19**, 131 (1924).

remains unexplored. Wendelmuth's investigation concerned the weakening of the jelly capacity of pectin solutions, sterilised and preserved under tetralin. Fruit juices and beet juice kept in this manner were examined weekly for viscosity. Beet juice gave anomalous results, but fruit juices gradually lost their jellying power, possibly because the pectic acid ester splits into free acid and methyl alcohol.

NON-SUGAR GELS OF PECTIN

Recently attention has been devoted to the production of non-sugar pectin gels, a subject having obvious importance in relation to the dietary of diabetics.

Gelation of the aqueous pectin system is brought about by the action of the enzyme pectase.[1] According to Paul and Grandseigne [2] milk can be made into a jelly by means of pectin and pectase. The enzyme acts best at 52°—55° C. and is but slowly destroyed at 70° C., but is rapidly destroyed at 80° C. A 1 per cent. pectin sol is easily set to a firm gel by a minute amount of the enzyme which is recovered from several plant juices. In neutral or very faintly acid solutions, containing alkaline earths, very good gels are obtained.

Mehlitz [3] has carried out extensive studies on the optimum conditions for the coagulation of pectin by pectase. The enzyme was prepared from the juice of fresh luzerne, 5 kg. being pressed to yield 1,600 c.c. juice. After preservation for 22 hours in the dark with $CHCl_3$ it was filtered and centrifuged, the final yield of clear juice being 970 c.c. To this was added double volume of 90 per cent. alcohol and the white precipitate was then freed from alcohol and taken up in 400 c.c. water. After 15 hours' standing the solution was filtered and precipitation again effected by pouring the solution as a thin stream into 90 per cent. alcohol. Drying *in vacuo* over $CaCl_2$ gave a powder which kept excellently, although the solution rapidly lost its enzymic potency.

The apple pectin used throughout the investigation was Pomosinextrakt KOL of composition :

	Per cent.
Total extract	7·5
Acid (as malic)	0·40
pH	4·04
Pectin (as Ca pectate)	3·40

To this was added 0·3 per cent. $CaCl_2$. The optimum pH for coagulation of the pectin by pectase was 4·8—5. From an initial series of

[1] See Willaman, *Univ. Minnesota, Studies Biol. Sci.*, **6**, 333 (1927).
[2] *Bull. assoc. chim. sucr. dist.*, **46**, 233, 245 (1929).
[3] *Biochem. Z.*, **221**, 217 (1930).

tests Mehlitz arrived at the following conditions for his standard gel :—

Pectase solution 10 per cent. . .	0·8 c.c.
Water	4·2 c.c.
Pectin solution	5·0 c.c.

This coagulated in $5\frac{1}{2}$ hours and the coagulum had the analysis :

	Per cent.
Pectase	0·8
$CaCl_2$	0·15
Ca pectate	0·85
pH	4·87

He now investigated as variants, $CaCl_2$, pectin and pH. With $CaCl_2$ it was found that the optimum amount was 0·15 per cent. in the coagulum. More or less $CaCl_2$ resulted in a longer setting time. The optimum Ca pectate content of gels was 1 per cent. whilst below 0·7 per cent. very poor gels were obtained. All the gels obtained were tasteless and similar to agar, gelatin or the usual pectin gels, but had a finer and more tenuous structure.

CHAPTER XI

COLLOID PHENOMENA IN BREWING

Beer is the fermented liquor prepared from malted barley and other cereals, hops being used for flavouring. In addition to the constituents given in the usual analyses [1] many other products of fermentation ensue, such as sugars, dextrin, proteins, tannin, and glycerin.

Although the brewing of beer is essentially a biochemical operation involving enzyme reactions and yeast growth, subjects beyond the scope of the present volume, there are certain important points which have much interest from the colloid-chemical standpoint. These include protein peptisation and flocculation and the important phenomenon of frothing or foaming.

Very briefly outlined, the preparation of beer involves the following stages :—

(1) The malting of barley : the grain is soaked in water, germination proceeds, and by the action of diastase some of the starch is converted into sugar. Enzyme action also breaks down nitrogenous substances.

(2) The malt is dried, crushed into fine particles or "grist," and mashed, *i.e.* heated with water to dissolve maltose and dextrins produced by enzyme action. The solution is known as sweet wort.

(3) The wort is sterilised by boiling for 1—2 hours, an operation leading to the coagulation of proteins. The wort is boiled with hops which contribute further colloid ingredients to the system.

(4) The strained wort is cooled and aerated and then fermented by yeast. The resulting beer is then clarified for consumption.

At all stages the pH influence is very marked, as the extensive literature thereon bears witness.[2] Such pH influence concerns the colloid changes and also the course of enzyme actions, yeast activity and bacterial infection. The pH value drops stage by stage, *e.g.* barley extract (pH 6·3—6·0), malt extract (pH 6·2—5·9), unboiled wort (pH 6·1—5·8), wort boiled for 3 hours (pH 5·8—5·5), and beer (pH 4·6—4·3).[3]

[1] Cox, "The Chemical Analysis of Foods" (London, 1926), p. 192 ; Grant, *J. Inst. Brewing*, **15**, 385 (1909) ; Remy, *Z. Untersuch. Lebensm.*, **59**, 402 (1930).

[2] Useful summaries are given by Britton, *Ind. Chemist.*, **4**, 150 (1928) ; Petit, *Brasserie malterie*, **17**, 241 (1927) ; von Laar, *Chimie et industrie*, **14**, 511 (1925) ; Hagues, *J. Inst. Brewing*, **33**, 262 (1927).

[3] Kolbach, *Wochsch. Brau.*, **47**, 145, 163 (1930).

COLLOID FACTORS IN BREWING

At every stage in the brewing of beer colloid factors predominate and an extensive literature has developed in this connection. Emslander [1] is a prominent pioneer worker in this connection as his many publications will show and he has recently succinctly summarised [2] his work and views. A little of the immediately following discussion follows in part his summary, adding thereto relevant details of others and of later work.

In the malt house commence the first colloid chemical reactions. The barley is steeped for 90 hours in water, the chemical composition of this playing an important *rôle* in relation to the type of finished beer.[3] Apparently the anions and cations influence the formation of diastatic and proteolytic enzymes according to the Hofmeister ion series. The pH value is particularly important in this connection, *viz.:* in the swelling and growth of the barley.[4] Just how the electrolytes of the water penetrate the barley [5] is problematical, as the chaff acts as a semipermeable membrane, but Emslander believes the water penetrates the sprouting grain, undergoes dialysis and the salts become adsorbed by the colloids present.

Experiment shows that the more difficultly soluble proteins are located in the chaff and that worts and beers prepared from pure malt grain do not exhibit protein haze,[6] whilst those prepared in the presence of chaff do.

Most investigators agree with Emslander that the chemical composition of the steep water greatly influences the quality of the malt.[7] Investigating the action of waters buffered over the range pH 4·5—7·0 shows that the steep water invariably becomes darker the more acid is the water. Similarly, water plays a considerable *rôle* in the leaching of hops, softened water necessitating more hops than hard (carbonate) water, and the pH value is again significant.

The germination of the barley leads to the formation of proteins, whence it follows that the reaction obtaining inside the grain has special importance since the peptising enzyme acts best in an acid

[1] *Z. physik. Chem.*, **49**, 317 (1904); *Kolloid-Z.*, **2**, 88 (1907); **2**, 308 (1908); **6**, 156 (1910); **7**, 177 (1910); **10**, 189 (1910); *J. Inst. Brewing*, **18**, 484 (1912); *Wochsch. Brau.*, **30**, 387 (1913); **33**, 169 (1916); *Z. ges. Brauw.*, **37**, 2 (1914).

[2] Liesegang's "Kolloidchemische Technologie" (Dresden and Leipzig, 1927), p. 816 *seq.* Emslander's summary should certainly be consulted by the student as a very readable account by a leading authority. A later summary will appear (in English) in the forthcoming Vol. III. of Alexander's "Colloid Chemistry" (New York).

[3] Lüers, *Wochsch. Brau.*, **44**, 385 (1927); Windisch, *ibid.*, **36**, 1 (1919); **43**, 423, 444 (1926); Rohland, *ibid.*, **27**, 309 (1910); Wustenfeld, *ibid.*, **27**, 361 (1910); Hermann, *Allgem., Z. Bierbrau. Malzfabr.*, **41**, 315 (1913); Reichard, *Z. ges. Brauw.*, **36**, Nos 25 and 26 (1913); Petit and Raux, *Brasserie malterie*, **16**, 289 (1926).

[4] Emslander, *Kolloid-Z.*, **14**, 44 (1914); Kolbach, *Wochsch. Brau.*, **47**, 145, 163 (1930); Windisch and Kolbach, *ibid.*, **44**, 237, 243 (1924); **46**, 345 (1929).

[5] Cf. Ling, *J. Inst. Brewing*, **34**, 75 (1928).

[6] Cf. Windisch, *Wochsch. Brau.* **44**, 25 (1927).

[7] Cf. Jalowetz, *Brau Malzindustrie*, **2**. 25 (1930).

medium and the tryptic enzyme in an alkaline medium. Should the temperature of the barley exceed 18° C., lactic acid forms in considerable amount and tryptic digestion is inhibited. Tryptases dissolve proteins to yield peptases, so that good practice is to conduct germination at the lowest possible temperature and to malt longer. Malt peptase acts best at pH 3·2 and malt tryptase at pH 6·4.

The influence of temperature on malting has been studied by Stadler [1] in his work on the effect of the protein content of barley on the quality of dark beer. The following table collects data relating to three malts prepared by Stadler and the beers made therefrom :—

TABLE 74. RELATION OF MALTING TO BEER QUALITY

Malts.				Beers.			
Floor Temp.	Total Protein.	Colour.	pH.	N_2 on Dry Extract.	pH.	Surface Tension.	Flavour and Body.
	Per cent.			Per cent.		dynes/cm.	
25·6° C. .	15·95	1·6	5·55	10·24	4·74	64·0	+ + +
19·3° C. .	15·80	1·4	5·54	10·73	4·75	64·0	+
Normal .	10·90	0·8	5·81	6·55	4·0	67·9	+

Just what is the reason why the elevated temperature in the case of the first sample should produce so pronounced a difference in the quality of the beer is not yet known, but it seems to bear some relation to the acceleration of germination and the high protein content of the barley.

As the changes which take place during the mashing of malted barley depend on the pH prevailing, the importance of the buffer content of malted barley will be realised. A thorough investigation into the formation of buffer substances during malting has been conducted by Hopkins and Kelly.[2] Some of the buffers derived from the malt are formed during mashing itself as a result of enzyme action, this in turn depending on the pH, already influenced by the buffers soluble in cold water.

The observations were restricted to corns from which all rootlets had been removed, and various barleys were used. It was found that : (*a*) about 50 per cent. of the buffering substances of the original barley are removed during steeping and the pH value falls owing to the extraction of such alkalies as phosphates ; (*b*) during the first two days on the malting floor little change occurs, but the maximum activity shows itself during the next four or five days, the buffer ratio in the " phosphate zone " increasing to two or two and a half times as much and one and a half times as much in the " amino

[1] *Wochsch. Brau.*, **46,** 479 (1929).
[2] *J. Inst. Brewing*, **35,** 402 (1929). Cf. Lüers, *Wochsch. Brau.*, **44,** 97 (1927).

acid zone." Meanwhile the pH value rose from about 5·1 or 5·2—5·8 or 5·9.

Similar but gradual changes occur from about seven days until about one day after loading on to the kiln. During the remainder of the kilning and curing process no pronounced change takes place other than the fall in pH value from 6·0—6·1 to about 5·7.

The student is recommended to study the authors' paper in detail, as interesting speculations are made relating to the association of the above phenomena with the actions of the several enzymes functioning in the growing corn.

After lying on the floor at a temperature below 18° C., for 7—12 days, enzymes form capable of splitting protein and carbohydrate. The malt kiln is now operated at higher temperature (up to 70° C.) for 48 hours. Proteins are dissolved and at the finishing temperature (80°—100° C.) there are formed the colloidal substances which produce the colour and aroma of beer, in particular of dark beer. Milling of the malt follows.

The next operation is conducted in the brewing house, where the proteins and more especially the carbohydrates undergo further decomposition and are brought into solution. Temperature and pH value become significant, a pH 4·5—5·0 being optimal.

Windisch and Kolbach [1] have studied the influence of the mashing process and pH on the composition of the wort and the acidity of the final beer. They prepared mashes with an alkaline liquor, neutral liquor and two acid liquors. The more acid mash gave a maximum malt extract of 79·9 per cent. and the neutral mash gave the minimum yield, 76·5 per cent. The pH value of the worts varied from pH 5·75 in the alkaline mash to pH 5·25 in the acid mash. The beer prepared from the neutral mash was more acid than the beer prepared from the acid mashes. A low surface tension of the mash is favourable to fermentation and a mash which "bumps" on boiling is difficult to ferment. In this connection, Emslander [2] holds the view regarding enzyme action, that it is incorrect to state that the optimum action of an enzyme lies at a definite pH value. Rather is the reaction optimum of an enzyme to be correlated with the specific surface tension of the substrate. The enzyme seeks to provide these constants, forming acid and attaining that pH involving a favourable surface tension.

Another important factor in the brewing house is the nature of the salts in the wash water, their influence, in Emslander's opinion, being not so much on the pH value, as on the course of the washing out of the malt constituents. He finds that worts arising from protracted leaching give a less stable beer, protein haze being a defect. This is because protracted leaching dissolves out proteins

[1] *Wochsch. Brau.*, **44**, 237, 243 (1924).
[2] *Z. ges. Brauw.*, **42**, 135 (1919).

for the decomposition of which no enzymes are typified or for which the enzymes available have feeble action.

The liquor containing the extractives from the malt is the wort, boiling of which can precipitate proteins,[1] irreversibly, the amount increasing with the time of boiling and the higher the pH value.[2] Thus, at pH 4·84 the precipitated protein is about 75 per cent. of that precipitated at pH 6·37. Lüers mentions pH 5·2 for optimum flocculation,[3] whilst other authorities [4] give the value pH 5·4.

Since wort contains colloidal ingredients, its surface tension is low. King [5] finds the surface tension of a wort of the standard favoured in this country in preparing high-quality beers (S.G. = 1·0726) as 41·14 dynes/cm. at 15·5° C. When the wort was concentrated to a density of 1·1793, its surface tension measured 41·05 dynes/cm.

Small quantities of surface-active ingredients must be responsible for the surface tension lowering, since the main constituents (brewing sugar, dextrin, maltose and caramelised sugar) do not effect marked lowering. Such surface-active materials concentrate in the froth produced when air is bubbled through wort and undergo coagulation. Then phenomenon is reflected in the data obtained by Peard and Johnston [6] who passed air bubbles at the rate of 1 per second through all malt wort (S.G. = 1,080°) at 15·5° C. The following surface tensions were measured :—

	Dynes/cm.
At commencement	48·2
After 5 mins. bubbling . . .	48·0
,, 15 ,, ,, . . .	48·2
,, 30 ,, ,, . . .	48·6
,, 60 ,, ,, . . .	49·0
,,1,080 ,, ,, . . .	58·2

Using a bright filtered wort diluted with distilled water to a density = 1039·2° and passing 3 air bubbles per second, it was observed that after 24 hours' bubbling the surface tension became 67·1 dynes/cm., as compared with the original value, 54·3 dynes/cm. The original nitrogen content was 0·0789 gr. per 100 c.c. and the final value was 0·0728 gr. per 100 c.c. A considerable deposit of solid material was observable on the sides of the beaker where the froth had collapsed.

As regards the influence of pH on the surface tension of wort,

[1] *Vide* Jalowetz, *Wochsch. Brau.*, **46,** 483 (1929).
[2] Windisch *et al.*, *ibid.*, **46,** 403, 417 (1929).
[3] Cf Lüers, *Z. ges. Brauw.* **53,** 55 (1930).
[4] Peard and Johnston, *J. Inst. Brewing,* **31,** 425 (1925); Windisch *et al.*, *Wochsch. Brau.*, **39,** 42 (1922).
[5] *J. Inst. Brewing,* **31,** 32 (1925).
[6] *Ibid.*, **31,** 416 (1925).

the authors found that the surface tension was a maximum at pH 5·4 and a minimum at pH 6·2.

Now follows the operation of boiling the wort with hops. The extracted hop resins act as protective colloids for the proteins, thus enhancing the frothing capacity of the beer and giving it "body." The hop tannin acts as a precipitant for the proteins and thus assists the subsequent stability of the beer. The pH value plays an important *rôle* in this precipitation. Intensive boiling with hops increases the dispersion of the hop resins.[1]

Colour develops during the boiling of wort, the intensity varying with pH. Thus, Windisch and Kolbach [2] observed that at pH 6·16 the increase in colour due to boiling for 2 hours was 100 per cent., whereas for pH 5·2 it reached only 30 per cent. increase. Independently of pH there was a time factor, the colour intensity rising proportionately with the duration of boiling up to 4 hours. Other factors involved in the development of colour are the character of the malt, the degree of aeration, and the hops present.

Hagues [3] has made an extensive research into the influence of pH in the wort boiling process. Cool sweet wort has a haze due to the presence of numerous very fine particles in suspension. On heating, the wort gradually clarifies, but on boiling, the haze reappears, subsequently coagulating to larger particles suspended in a clear wort. Sedimentation soon removes these particles permitting filtration in the brewery in the hop-back. This coagulation is frequently termed the *break*,[4] although some brewers include in this definition the total coagulation given on boiling and the subsequent cooling.

The author's results show that for pH values more alkaline than pH 4—5, the quantity of protein coagulated during boiling is independent of the pH value. When the pH is less than 4·0, less protein matter is coagulated, possibly because of acid hydrolysis or the formation of a soluble protein-acid salt. The coagulation of protein and the accompanying fall in pH are gradual changes detectable even at 71° C.

The size of the particles of coagulum is independent of the pH value above pH 5·0, but is very susceptible to pH influence for values less than pH 5·0. The lower the pH value to which the original wort is adjusted, the more finely divided is the coagulum in the boiled wort. A slightly larger coagulum particle is obtained with more concentrated worts, and by aeration or agitation. Hops were found to be without influence on the size of the particles of the coagulum.

[1] But see the contrary results of Horsch and Schulteis, *Wochsch. Brau.*, **46,** 295 (1929).

[2] *Ibid.*, **44,** 53, 64 (1927).

[3] *J. Inst. Brewing*, **33,** 262 (1927).

[4] Cf. Brown, *ibid.*, **19,** 84 (1913); Moritz., *ibid.*, **27,** 565 (1921); **29,** 464 (1923).

Table 75 collects some of the author's results, boiling being conducted for 2 hours and sedimentation at 20° C.

TABLE 75

First Length Brewery Wort Unhopped.			First Length Brewery Wort Hops added : 2·08 gr./litre.	
Initial pH (adjusted).	Final pH.	Diameter of Particles.	Initial pH (adjusted).	Diameter of Particles.
		cm.		cm.
5·11	4·99	0·0121	2·1	0·0026
4·76	4·67	0·0118	3·5	0·0062
4·42	4·43	0·0109	5·1	0·0135
4·14	4·18	—	6·6	0·0141
3·92	3·98	0·0093	7·7	0·0136

After the hop-boiling process, the malt-hop wort is cooled and agglutination and sedimentation of proteins occur. Maintaining the wort in a constant rolling motion during cooling assists the conglomeration of the fine coagula, and sedimentation proceeds more effectively. The manner of cooling has particular importance in view of the subsequent fermentation after the wort is filtered.

The important operation of fermentation, wherein yeast acts on the maltose, dextrin, proteins and other substances in the wort introduces several colloid-chemical reactions. The acidity rapidly increases and after a few hours the pH value attains the optimum for yeast action, *viz.* : pH 4·2 for worts from gypsiferous waters and pH 4·5 for worts from carbonate waters.[1] In the yeast cells significant electrical changes take place. Thus, Lüers and Geys [2] observed that resting washed yeasts carry a positive electric charge in worts, but after some hours when increasing cell action occurs, the yeast takes on a negative charge, reverting 5 days after fermentation to a positive charge.

At the onset of fermentation the yeast carries a positive charge, but during the course of fermentation the charge and, therefore, the stability of the yeast proceeds to an optimum value, coincident, according to Emslander, with a minimum surface tension. The conclusion of the fermentation finds the yeast carrying a positive charge again, the beer colloids isoelectric, and the surface tension low. The conditions favour the agglutination of the yeast, and yeast flocculation is a practical sign of the termination of fermentation.

Interesting research relating to the electrical charges on yeast has been conducted by Stockhausen.[3] Suspensions of clean bottom-fermentation yeast in water usually react as pH 6·5 and by

[1] Emslander, *Z. ges. Brauw.*, **42,** 127 (1919). See also the comprehensive work of Hagues, *J. Inst. Brewing*, **34,** 362 (1928).

[2] *Kolloid-Z.*, **30,** 372 (1922).

[3] *Wochsch. Brau.*, **44,** 121, 133 (1927).

cataphoresis can be shown to be positively charged. The yeast is here in the powdery or non-flocculating condition. On reducing the pH value, the positive charge diminishes and becomes zero at pH 3·8, where maximum flocculation of the yeast cells is noted. At still lower pH values the sign of the charge is negative. In wort, however, the colloid ingredients complicate matters as the yeast flocculation may occur at higher pH values owing to the cells adsorbing negatively charged colloids. In illustration may be noted that flocculation occurred at pH 6·3 in hopped worts, at pH 7·1 in worts boiled without hops, and at pH 7·3 in unboiled wort.

The practical importance of these observations lies in the fact that flocculation of the yeast would mean its early deposition with the consequence that attenuation was not carried sufficiently far. Top-fermentation yeasts were found to be less subject to electrical factors. In aqueous suspension they carried a small positive charge and their isoelectric range was pH 4—6.

Surface tension has been observed to play an important part in fermentation.[1] The poorer the wettability of the walls of the fermentation vessel, the higher is the surface tension of the fermenting wort and the greater the degree of attenuation reached. Under comparative conditions, the degree of attenuation reached 52·9 per cent., 57·4 per cent., 68·4 per cent., and 72·8 per cent. in vats of enamelled iron, lacquered, pitch-lined, and of non-wetted wood respectively. Scalding wooden vats prior to introducing the wort causes the disappearance of the air in the pores, and the wort adheres better to the walls with consequent lowering of the degree of attenuation. In recent years the large fermentation vats are made of aluminium, enamelled iron, or cement coated with pitch or paraffin wax. Enamelled iron is very good and permits an exceptionally favourable fermentation, whereby the foam tendency and the body of the beer are improved.

A similar surface effect has been noted in the flocculation of yeast and the coagulation of protein. Emslander and Freundlich found that the degree of attenuation runs parallel with the degree of clarification. Fermented beer became clear after 5 days in a paraffined vat, 6 days in a wood vat, and not after 8 days in a glass-lined vat.[2] The explanation advanced by Emslander is based on electrostatic influences, *e.g.*, enamel or glass carries a positive charge and repels the positively charged CO_2 bubbles, whilst paraffin with its negative charge attracts and holds the bubbles, thus acting as an accelerating influence in fermentation. The problem of beer clarification has analogous influences, the walls in part attracting and in part repelling the colloid ingredients.

[1] Emslander and Freundlich, *Z. physik. Chem.*, **49,** 317 (1904); Emslander *Wochsch. Brau.*, **46,** 401 (1929).

[2] Klinger, *Die Naturwissenschaften*, **5,** 193 (1917), reports similar surface effect in the coagulation of blood.

THE STABILITY OF BEER IN STORAGE

The problem of retaining a brilliant clarity in beer, especially cooled beer, during storage or transport has obvious commercial importance and its consideration introduces points of great interest to the colloid chemist. An unstable beer may develop turbidity or haze, and even throw down a more or less flocculent precipitate. The total solid matter involved is only of the order of 1 in 100,000.

No one cause is indicted in this. Numerous causes have been mentioned such as fats [1] derived from malt, wild yeasts,[2] bacteria,[3] proteins precipitated by electrolytes derived from the glass bottle,[4] protein combined with fluoride preservative,[5] calcium oxalate derived from hops,[6] silicic acid [7] which constitutes 25 per cent. of the total ash of the coagulum obtained by heating beer, nitrogenous matter,[8] caramel [9] and starch.[10]

Although all these causes of haze in beer may occasionally arise, undoubtedly the major factor concerned in the development of haze is desolution of protein matter. The earlier work of Emslander [11] using cataphoresis showed that hazy beer contained negatively charged colloids, the beer exhibiting pronounced turbidity on the anode side and bright solution on the cathode side of the U-tube. It is known also that the prolonged digestion of wort raises the pH value and reduces the stability of the proteins. Lüers [12] by experiments on anaphylaxis proved that protein haze contains proteins of which a considerable proportion are derived from the yeast.

The pasteurisation of beer impairs its subsequent stability and in this hop tannin [13] plays a part. Thus, Visez [14] believes the haze to be a complex of protein and tannin in a delicate equilibrium readily disturbed by heat and by electrolytes dissolved from glass. Tannin from the cork may also be a factor. Pasteurisation disturbs the

[1] Matthews, *J. Inst. Brewing*, **31**, 69 (1925).

[2] Christoph, *Z. ges. Brauw.*, **44**, 119, 127, 135, 147, 153 (1921); Will, *ibid.*, **40**, 249 (1917); Lindner, *Wochsch. Brau.*, **35**, 225 (1918); Petit, *Brasserie malterie*, **20**, 209, 225 (1930).

[3] Amberger, *Wochsch. Brau.*, **40**, 92 (1923).

[4] Disez, *ibid.*, **40**, 115 (1923).

[5] Will, *Z. ges. Brauw.*, **37**, 357 (1914).

[6] Bau, *Wochsch. Brau.*, **35**, 31, 40, 45, 51, 57, 63, 70 (1918); Geys, *Z. ges. Brauw.*, **45**, 2, 9 (1922).

[7] Netscher, *Wochsch. Brau.*, **45**, 582 (1928); **46**, 335 (1929); Kolbach, *ibid.*, **43**, 123 (1926). Petit, *Brasserie malterie*, **19**, 353 (1930).

[8] Bekart, *Petit J. Brass.*, **34**, 1542 (1926); *Chimie et industrie*, **19**, 415 (1928); Lind, *J. Inst. Brewing*, **37**, 17 (1931).

[9] Hancock, *Brewers J.*, **51**, 167 (1915); Harman and Oliver, *J. Inst. Brewing*, **31**, 577 (1925); Delroisse, *Petit J. Brass.*, **32**, 636 (1924).

[10] Windisch, *Wochsch. Brau.*, **33**, 105, 121 (1916).

[11] *Ibid.*, **33**, 170 (1916).

[12] *Z. ges. Brauw.*, **45**, 159 (1922).

[13] Hartong, *Wochsch. Brau.*, **47**, 295 (1930); Bilgram, *ibid.*, **46**, 137 (1929); Heintz, *ibid.*, **34**, 221 (1917); Windisch, *ibid.*, **35**, 310, 316, 322, 330, 335 (1918); Kolbach, *ibid.*, **47**, 145, 163 (1930); Comrie, *J. Inst. Brewing*, **36**, 307 (1930); Emslander, *Petit J. Brass.*, **36**, 1292 (1928); Reichard, *Kolloid-Z.*, **10**, 209 (1912).

[14] *Wochsch. Brau.*, **39**, 33 (1922); Hartong, *ibid.*, **47**, 295 (1930); Fink and Riedel, *ibid.*, **47**, 491, 501, 511 (1930).

colloid-chemical equilibrium of cooled and filtered beer owing to its increasing the grain-size of the colloid particles and so increasing their tendency towards flocculation. Obviously, it is desirable to regulate the pH value [1] to be as far removed as possible from pH 4·2, the isoelectric point of the beer colloids.[2] Alternatively, beer may be clarified at the isoelectric point.[3]

Interesting results have recently been obtained by Fink and Wildner [4] on the effect of adding tannin to unhopped 18 per cent. worts and to pale Munich beers. Of the total nitrogen in the wort, 9·57 per cent. was coagulable. Addition of 1/9 volume of 1 per cent. tannin solution gave on 3 days standing in a cool place a precipitate and analysis showed that of the original 9·57 per cent. of coagulable nitrogen 8·33 per cent. had been precipitated, leaving only 1·24 per cent. in solution. In other words, about 87 per cent. of the coagulable nitrogen of wort is precipitable by tannin under these experimental conditions. Of the original albumose nitrogen 35 per cent. was precipitated and of the original peptone nitrogen 49·5 per cent. was precipitated. Of the original total nitrogen in the wort, almost 21 per cent. was flocculated with tannin.

Quite different results were given in the case of the Munich beers. For example, using 1/9 volume of 0·25 per cent. tannin solution, 11 per cent. of the original total nitrogen of one beer was precipitated on standing for 6 days. Here, however, 77·4 per cent. of the nitrogen removed from solution was albumose nitrogen. No peptone nitrogen was precipitated, whilst coagulable nitrogen only accounted for 14·45 per cent. of the total precipitated nitrogen.

Various suggestions have been made as to methods for preventing haze in beer. Investigating the flocculation of protein-tannin complexes during the boiling of wort, Hartong [5] found that increasing the turbulence of boiling brings about greater flocculation of the complexes and removes in this fashion all the hop tannins. As only malt tannins remain in solution, the beer is more highly chill-proof. Ash [6] proposed the addition of a small quantity of tannin with SO_2 to beer so as to precipitate protein matter. Removing in this way about 31 mg. per litre of colloid matter, haze-tendency was reduced without impairing the foam-tendency of the beer.

The removal of the troublesome colloids by adding enzymes such as papain or the crude enzymes from germinating barley [7] to the beer before bottling or casking is known as the Wallerstein [8] process

[1] Van Cauwenberghe, *Chimie et industrie*, **19**, 415 (1928).
[2] De Gronckel, *Bull. assoc. élèves inst. sup. fermentations Gand.*, **29**, 335 (1928); Verbrugghen, *ibid.*, **30**, 354 (1929).
[3] Vanlehrberghe, *ibid.*, **28**, 322 (1927).
[4] Fink and Wildner, *Wochsch. Brau.*, **47**, 11, 23 (1930).
[5] *Ibid.*, **46**, 543 (1929); cf. Jalowetz, *ibid.*, **46**, 483 (1929).
[6] *Western Brewer*, **50**, 93 (1919).
[7] Meyer, Ger. Pat. 494,700 (1927).
[8] Brit. Pat. 12,350 (1911). *Vide* also Dutch Pat. 14,494 (1923).

and is very effective. The proteins may also be removed by suitable adsorbents.[1] The use of the super-centrifuge in the initial filtration of beer proved disappointing.[2]

Instead of attempting to remove the troublesome proteins, Visez [3] suggested the possibility of protecting the protein against precipitation by adding the protective colloid dextrin. Experiment showed that a high dextrin : N ratio reduces haze-tendency. Thus, a beer with a dextrin : N ratio of 3·4, after being bottled, pasteurised and chilled became turbid in 30 minutes, whilst another sample wherein the ratio was 8, took 300 minutes to develop haze.

BEER HAZE AND THE RAMSDEN PHENOMENON

There is another aspect of the haze problem, *viz.* the coagulation of the beer colloids at the air/liquid interface. As early as 1886, Bauer [4] showed that the transport of bottled beer resulted in the development of haze and staleness of flavour in those beers in bottles only poorly filled, whilst full bottles were in sound condition.

The clouding of liquids like beer and herb vinegars by agitation is an instance of the Ramsden phenomenon (p. 390) and is due to the adsorption and consequent desolution of colloid material at the air/liquid interface. The greater the agitation, the more extensive is the froth boundary exposed and the better the adsorption condition.[5] Emslander [6] has carried out some experiments in this connection and finds it advantageous to use bottles with long necks, exposing only a small air surface. He compared the intensity of the Tyndall cone in ordinary beer, the same beer well shaken, and beer to which 0·2 per cent. Na_2CO_3 was added. The transparencies were 3, 7 and 9 respectively, and haze appeared after the lapse of 38, 15 and 5 days respectively.

Under ordinary storage conditions, there is a definite time factor for the influence of the Ramsden phenomenon, as the colloid substances are only slowly adsorbed and denatured by the small surface exposed. Long maturing in casks would favour surface adsorption and subsequent filtration prior to bottling would give a chill-proof beer. However, micro-biological objections obtain in this connection.

The pressure of gas in a beer also influences haze tendency.[7] Thus, Hanow [8] filled beer into bottles : (A) direct from the storage vat,

[1] Hesselberger, *Wochsch. Brau.*, **46,** 285 (1929).
[2] Petit and Raux, *Brasserie malterie,* **16,** 276 (1926).
[3] *Wochsch. Brau.*, **39,** 73 (1922).
[4] *Z. ges. Brauw.*, **9,** 212 (1886). See also Serviere, " Der Scheidekunstler im Brau. u. Brennhause " (Frankfurt, 1816), pp. 137, 199.
[5] Cf. Nathan, *J. Inst. Brewing,* **36,** 539 (1930).
[6] *Kolloidchem. Beihefte.*, **3,** 47 (1912). See also Peard and Johnston, *J. Inst. Brewing,* **31,** 420 (1925).
[7] Emslander, *loc. cit.*, p. 52.
[8] *Wochsch. Brau.*, **11,** 349 (1894).

(B) after placing in casks. On standing at room temperature, (B) showed haze and sediment after 9 days, whilst (A) was still clear after 2 weeks. The CO_2 content per litre of the two original beers was (A) 3·29 gr. and (B) 2·34 gr.

SURFACE TENSION OF BEER

Peard and Johnston [1] have published data relating to the influence of pH on the surface tension of beer, which bears on the problem of haze. Fig. 54 is reproduced from their paper and the

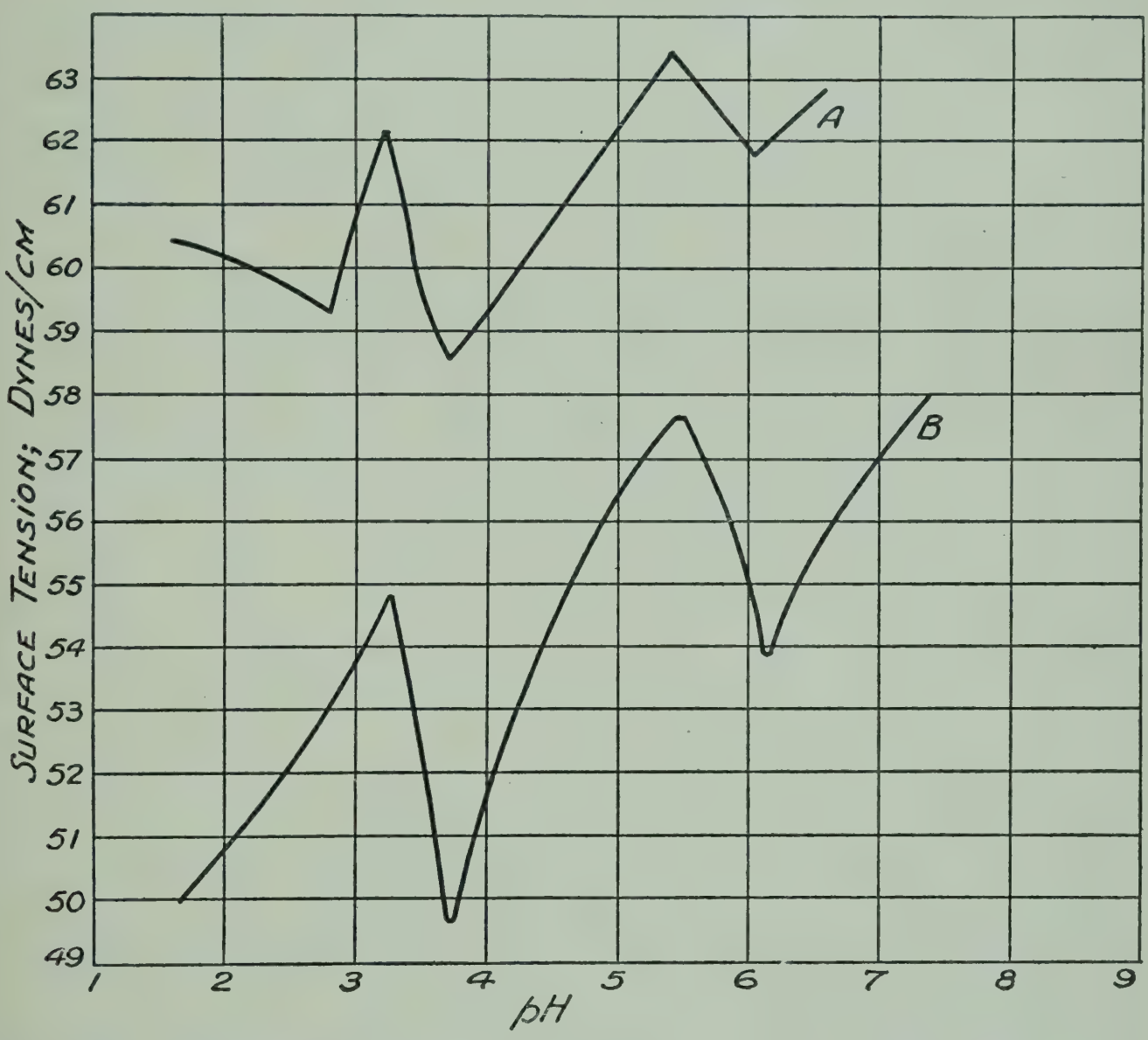


FIG. 54.—The surface tension of beers in relation to pH value.

graphs refer to (A) chilled and filtered pale ale (original gravity 1047) and (B) pale ale (original gravity 1047). Maximum surface tension values appear at pH 3·2 and pH 5·4 and a minimum value appears at pH 3·7. In the case of the chilled and filtered beer another minimum value for the surface tension is exhibited at pH6·0, whilst the second minimum in the case of the naturally matured beer is at pH 6·2. Chilled and filtered beer exhibits a further minimum at pH 2·7.

The beers at about pH 6·4 are hazy, due to incipient precipitation. As the pH rises, precipitation becomes abundant and at pH 8·0 extensive precipitation is seen. It is concluded that the surface

[1] *J. Inst. Brewing*, **31**, 422 (1925).

tension depressing agent in beer has its isoelectric point at pH 5·4 at which point the beer has poor stability. The incidence of precipitation at pH values higher than pH 5·4 is due to chemical changes consequent upon the alkali added in adjusting the pH value.

The authors point out that at pH 3·7 beer has a maximum stability as related to its colloid content, and a progressive decrease in stability will occur as the pH rises to pH 5·4. The normal pH of beer is 4·2.

FROTH OR FOAM

Froth or foam is the more or less stable extended air/liquid interface arising when bubbles persist at the surface of a liquid due to agitation, aeration or ebullition. An enormous film-surface of liquid is presented to the gas phase, so that foam is primarily a labile system owing to the tendency for the interface to be reduced to a minimum.[1]

Evanescent foams are formed when certain aqueous solutions of inorganic compounds are agitated. Thus, a 3 per cent. NaCl solution shaken in a bottle produces a temporary foam, as does also 97 per cent. commercial H_2SO_4. Again, certain organic liquids such as acetic acid will permit frothing in aqueous solution. *No pure liquid will yield a persistent froth*, cessation of agitation leading to the immediate subsidence of those bubbles which may have formed.

Plateau [2] recognised that in bubbles and foams the two chief factors necessary are a certain degree of viscosity of the liquid used and a low air/liquid surface tension. The former tends to resist thinning of the liquid films to the rupture point, whilst a low surface tension is necessary, since surface tension is the chief active force promoting thinning. Soap solutions readily yield permanent foams owing to their low surface tension and their high viscosity. Plateau defined two viscosities for a liquid capable of frothing, the *internal viscosity* due to internal friction and the *superficial viscosity* which resists the movement of a body situated in the air/liquid interface. Stables and Wilson confirmed the existence of this superficial viscosity in the case of a saponin solution which frothed readily.

Hillyer [3] determined the surface tension and foaming capacity of a number of solutions in an approximate manner and correlated the

[1] For detailed discussions on various aspects of foams see Desch, *J. Inst. Metals*, **11,** 57 (1914); **22,** 241 (1919); Boys, "Soap Bubbles" (London, 1924); Wells, "Surface Films" (London, 1930); Clayton, *Ind. Chemist.*, **1,** 489 (1925); Foulk, *Ind. Eng. Chem.*, **21,** 815 (1929); Ostwald and Meissner, *Kolloidchem. Beihefte*, **26,** 1 (1928); Rehbinder and Wenstrom, *Kolloid-Z.*, **53,** 145 (1930); Edser, *Brit. Assocn. Colloid Repts.*, **4,** 314 (1922).

[2] *Pogg. Ann.*, **141,** 44 (1870).

[3] *Phil. Mag.*, (5), **15,** 406 (1883). Cf. Luvini, *ibid.*, **40,** 190 (1870); Quincke, *Wied. Ann.*, **35,** 589 (1888); Rayleigh, *Proc. Roy. Inst. Gt. Brit.*, **13,** 85 (1890); Schutt, *Ann. d. Physik.*, (4), **13,** 712 (1904); Johlin, *J. Biol. Chem.*, **84,** 543 (1929).

results with the viscosities of the solutions. He noted that high viscosity alone will not account for stable foams : the surface tension must be low. Thus, 50 per cent. aqueous glycerine or a 6 per cent. aqueous solution of gum acacia, each very viscous, but with relatively high surface tensions, do not yield persistent froths, whilst beer and milk, not so viscous, but with lower surface tension values, froth easily.

The more persistent froths result when aqueous solutions of such compounds as saponin, soap, proteins, rennet, etc, are agitated and in each instance it can be demonstrated that the solute markedly reduces the surface tension of water. There is, however, no relation between the surface tension of a liquid and the *size* of the bubbles obtained therefrom, but the viscosity is a definite factor in this connection.[1] The explanation of frothing is based on surface tension considerations.

SURFACE TENSION AND FROTHING

The Gibbs equation connecting the surface excess of solute (Γ) at an interface with the surface energy is

$$\Gamma = -\frac{c}{\mathrm{RT}} \cdot \frac{d\sigma}{dc}$$

where σ = surface tension, and c = equilibrium concentration of solute in solution

Edser [2] has pointed out that frothing accompanies the agitation of a solution when $-\frac{d\sigma}{dc}$ has a finite value, negative or positive. For 3 per cent. NaCl solution, to instance an evanescent froth, $d\sigma/dc$ is positive and small and for $-c\,d\sigma/dc$ to be finite, c must be large. For those solutes which enhance stable forms, $d\sigma/dc$ has a large negative value, so that $-c\frac{d\sigma}{dc}$ is large, although c may be very small.

Accordingly, since the coalescence of bubbles in a liquid occurs when the bubbles approximate close enough so that the liquid between them is sucked out, such coalescence cannot be avoided in a pure liquid. Some solute must be present, and even then $-c\,d\sigma/dc$ should not be very small or equal to zero, since this would produce conditions similar to those obtaining in pure liquids. It is obvious that, in order to avoid coalescence of the bubbles, the constitution of the solution must be different in the space separating the bubbles to that in the main bulk of the solution. Such a state of affairs

[1] Schnurmann, *Z. physik. Chem.*, Abt. A., **143,** 456 (1929) ; cf. Weichherz, Bodea and Nord, *ibid.*, **150,** 1 (1930).

[2] *Loc. cit.*

only obtains when $-c\,d\sigma/dc$ has a finite value, positive or negative. As Bancroft [1] emphasises, the *sine qua non* for foam formation is the existence of a distinct surface film concentration gradient, *i.e.* the concentration in the surface layer must differ perceptibly from that in the bulk of the liquid. Either negative or positive adsorption must occur at the air/liquid boundary or bubble walls.

In the case of two-phase foam systems one usually is concerned with aqueous solutions of organic reagents which strongly depress the surface tension of water, and it has been shown that the curve connecting foam-stability with concentration of solute passes through a maximum. Temperature influences foam duration, increase in temperature acting adversely.[2]

The fact that solutes which strongly depress the surface tension of water yield the most stable foams is connected not only with the large negative value of $d\sigma/dc$ but frequently with certain irreversible changes in the solute due to its adsorption at the air/liquid interface. The surface film around the bubbles is mechanically strengthened either by a pronounced viscosity factor or by an actual structural factor. Stability of the film is often assisted by the presence of small solid particles.

Although many workers [3] have dealt with the problem of foams, Ramsden's investigations stand out in importance, particularly in relation to technical foams.

INTERFACIAL ADSORPTION

Ramsden [4] showed that agitation of various proteid solutions caused a separation of some of the solute in the form of fibrous or membrano-fibrous solids and, in some cases, *e.g.* egg-albumin " by purely mechanical means these free-surface coatings can be heaped up to form visible solid masses of proteid which in some cases is not only ' de-soluted,' but at the same time coagulated and rendered permanently insoluble in the mother-liquid." He found that such " surface-coagula " could be separated from many proteid solutions [5] and from certain colloidal solutions and suspensions, even when the concentration of solute was very small, in fact only 1 in 1,000,000. The separated solid possessed either a delicate membranous structure, a fibrous structure, or loosely contiguous particles.

Ramsden [6] observed in such solutions a typical Plateau surface

[1] " Applied Colloid Chemistry " (New York, 1926), p. 364.
[2] Cf. Behrman, *Ind. Eng. Chem.*, **21**, 818 (1929).
[3] Hall, *Proc. Roy. Soc.* (*Dub.*), **9**, 56 (1899); Benson, *J. Phys. Chem.*, **7**, 532 (1903); Zawidzki, *Z. physik. Chem.*, **35**, 77 (1900); **42**, 612 (1903); Metcalfe, *ibid.*, **52**, 1 (1905); Rhode, *Drude's Ann.*, **19**, 935 (1906).
[4] *Arch. Anat. Physiol.*, 517—534 (1894); *Z. physik. Chem.*, **47**, 336 (1904); *Trans. Liv. Biol. Soc.*, **33**, 3 (1919).
[5] See Schmidt, *J. Hyg.*, **14**, 399 (1914).
[6] Cf. *Trans. Faraday Soc.*, **26**, 796 (1930).

viscosity, accompanied by a special superficial resistance to shear. "The molecules which stud the surface are in such close lateral continuity that they make it tangentially 'rigid.'" The time factor varied greatly with the type of system investigated. His earlier work records that "bubbles of solutions of egg-albumin, caseinogen and saponin exhibit remarkable phenomena, which show that the bubble film as a whole is very imperfectly elastic and is covered with solid membranes. Egg-albumin bubbles are deformed on collapse by the formation of persistent folds of solid proteid in the bubble film. Bubbles of pure saponin solution, containing 0·01 per cent. or more of saponin, fall on collapse into innumerable shimmering folds containing isolated curved rods of *solid* saponin, although water is capable of dissolving at least 2,500 times this amount."

Obviously such solid matter must raise the stability of a bubble. A coherent membrane is not essential as solid particles will also stabilise bubbles, since, as Ramsden has pointed out, they (1) serve as *points d'appui*, (2) oppose local disturbances of the film, (3) oppose such deformation of the surface as tends to expose a new surface with higher surface tension.

It was recognised by Ramsden and confirmed by later workers [1] that his mechanical surface-coagula are the result of adsorption of solute at the air/liquid interface, with adsorbed molecules passing partly out of solution. Adsorption-surfaces which yield solid "massed adsorpta" are either extremely viscous (or even rigid), in which case the adsorbed particles are in direct mutual contact, or they are freely mobile, the particles being then more widely spaced. Saponin and proteins yield rigid adsorption-surfaces, whilst quinine and soaps yield typical freely mobile adsorption-surfaces. "In the many cases where adequately durable bubble-films are obtainable, the interference colours developed as the film gets thinner are disposed in horizontal bands when the surfaces are mobile, but are disposed chaotically when the surfaces are rigid."

Although most "massed adsorpta" are completely resoluble, some such as egg-albumin and fibrinogen remain insoluble, a change "attributed to catalytic condensation of the close-packed specifically strained and orientated protein molecules into a single vast chemical aggregate."

In those systems where there is a surface-rigidifying solute and a non-rigidifying solute capable of exercising a greater surface tension depression, the free surface is mobile, owing to the preferential adsorption (independent of concentration) of the more surface-active solute.[2]

[1] See especially Wu and Ling, *Chinese J. Physiol.*, **1**, 407 and 431 (1927).
[2] Cf. Rehbinder and Wenström, *Kolloid-Z.*, **53**, 156 (1930).

Wilson and Ries [1] have published most interesting data relating to surface films and their rigidity. They point out that the collapse of a bubble results from the tendency of the solution to run down the bubble surface and thin out the film to breaking point and hence some stabilisation of the film is required to resist the force of gravity or to prevent thinning by capillary forces. Film formation was studied at the air/liquid interface with soaps, saponin and other substances. A torsion pendulum apparatus was employed to measure accurately the superficial viscosity of the films. The results obtained with sodium stearate in aqueous solution showed: (i.) surface films even in the case of a 1 in 100,000 dilution are plastic solids and not viscous liquids, (ii.) a time factor enters into film formation, (iii.) higher concentrations of the soap build up films more rapidly and eventually give stronger films.

Sodium oleate gave evidence of marked adsorption at the air/liquid interface, but only in the case of more concentrated solutions ($>$ 1 in 5,000) was a plastic solid film formation made evident. Saponin showed a much better tendency to form films. The gradual growth of surface films was investigated by an ultramicroscopic analysis and the final thickness observed in the different solutions varied from 10 to 40 microns.

The experimental data was correlated with tests of foam-stability and the conclusion reached that the main factor in producing persistent bubbles is not the surface tension [2] or the viscosity of a solution but the presence in the films of a "plastic solid surface," an opinion shared by Freundlich.[3]

The phenomenon is not confined to solutions of organic substances; the recent work of Stark [4] shows that ferric oxide sols are coagulated by bubbling gases or by mechanical agitation. The great gas/liquid interface leads to adsorption of the colloid and "a concentration of particles beyond the limits of stability takes place and in this way brings about coagulation."

TWO-PHASE AND THREE-PHASE FOAMS

Bartsch [5] has carried out a very extensive investigation on foam systems. He found the significant fact that in two-phase foaming systems, the duration of the foam depends on the concentration of foaming agent present, this duration being a distinct maximum at low concentrations. Thus, taking the fatty acid series as an example, Bartsch gives the following data:

[1] *Am. Coll. Symp. Monog.*, **1,** 145 (1923); also du Noüy, J. *phys. radium*, **6,** 145 (1923), and *Compt. rend.*, **174,** 962 (1922); Horn, *Am. J. Pharm.*, **98,** 53 (1926).
[2] Ostwald and Steiner, *Kolloid-Z.*, **36,** 342 (1925).
[3] "Colloid and Capillary Chemistry" (London, 1926), p. 790.
[4] *J. Am. Chem. Soc.*, **52,** 2730 (1930).
[5] *Kolloidchem. Beihefte*, **20,** 1 (1925).

TABLE 76. OPTIMUM FROTH-FORMATION OF FATTY ACIDS IN WATER

Acid in Solution.	Maximum Duration of Froth.	Concentration of Acid at this Maximum.	Surface Tension of Solution at this Maximum.
	Seconds.	Mol./litre.	Dynes /cm.
Formic . . .	4	0·45	70
Acetic . . .	8	0·20	69
Propionic . .	11	0·25	61
Butyric . .	18	1·0	33
Valeric . . .	9	0·015	64
Capronic . .	13	0·0075	57
Heptylic . .	16	0·0015	60
Caprylic . .	12	0·00025	66
Nonylic . .	5	0·00007	70

From these and many other results it is evident that the maximum persistence of froth is greater, the lower the surface tension of the solution exhibiting maximum frothing. In the case of concentrated solutions the influence of surface activity is not supreme, as solubility and degree of dispersion must be considered. For example, the higher members of homologous series of surface-active substances possess smaller foam-forming capacities than the middle members. The higher the member in the series, the greater is the content of the solution in respect to submicrons, and submicrons alone are unfavourable to foams.[1] Increasing the *molecular* solubility of a surface-active substance raises its foaming power, *e.g.*, dissolving nonylic acid in 60 per cent. aqueous ethyl alcohol.

Bartsch emphasises that solutions of surface-active substances give better foams, the steeper the concentration gradient between the Gibbs adsorption layer and the solution and the greater the heterogeneity of this layer.

Dealing with the presence of solid particles entangled in foams (three-phase systems) Bartsch shows that the stability of the foam depends upon (1) the concentration of the frothing agent, (2) the affinity of the frothing agent and the solvent for the solid phase, (3) the degree of dispersion of the solid phase. Regarding the latter factor, he states that solids of fine dispersion enhance the duration of foam much less than do solids of much coarser dispersion. In the dispersion range between colloidal solution and coarse suspension there is an optimum degree of dispersion corresponding to maximum foam persistence.[2]

[1] Cf. Traube and Klein, *Kolloid-Z.*, **29,** 236 (1921).

[2] Alexander speaks of a zone of maximum colloidality, *J. Am. Chem. Soc.*, **43,** 434 (1920); *Kolloid-Z.*, **36,** 334 (1925).

Ostwald and Steiner [1] expressed the view that there is no necessary relation between foaming power and reduction of surface tension, and to this Bartsch [2] takes exception. Determining the surface tension curves and foam stability-concentration curves for certain alcohols and fatty acids in aqueous solution (Fig. 55), he finds that foam stability increases to a maximum (coinciding with maximum depression of surface tension) and then rapidly decreases, *i.e.* foaming power diminishes completely at sufficient concentration of the surface-active solute. Bartsch explains this on his heterogeneity theory which correlates maximum foaming with equal numbers of

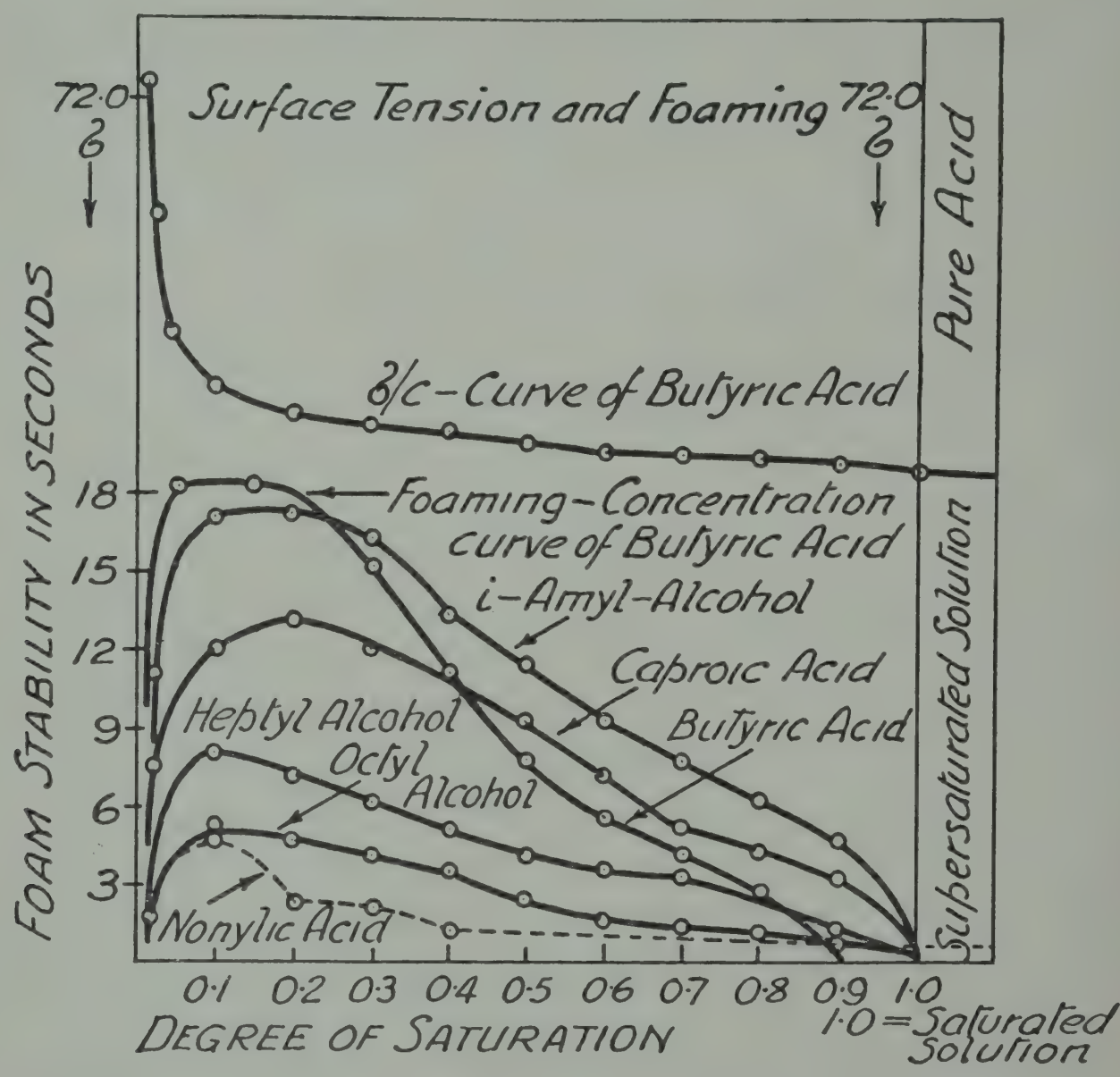


FIG. 55.—Relation between surface tension and foam stability.

solvent and of solute molecules in the surface layer. Steep concentration gradient is fundamental to the formation of foam and subsequent heterogeneity, *e.g.*, by the presence of de-soluted material, increases stability. It is necessary to bear in mind the conditions necessary for foam formation and the conditions influencing the *properties* of the foam, *e.g.*, its stability. Thus, sodium cholate solutions yield a foam and its stability may be enhanced by solids. Addition of (say) $CHCl_3$, which wets the solid better than does water, will cause collapse of the foam.

[1] *Kolloid-Z.*, **36**, 342 (1925).
[2] *Ibid.*, **38**, 177 (1926).

SAPONIN FROTHS

Saponin solutions exhibit remarkable frothing powers, the extent of the frothing depending on the kind of saponin used.[1] The subject carries interest owing to the former practice of using saponin in beverages as a frothing agent (now recognised as physiologically undesirable) and because of its recent investigation in connection with the theory of frothing.

Several workers have determined the surface tension of aqueous saponin solutions,[2] such determinations being complicated by skin formation at the surface.[3] An important investigation by Rehbinder and Wenström [4] dealt with the stability of the bubbles produced with saponin, connecting this with the degree of adsorption of saponin at the bubble walls. Table 77 is reproduced from their report.

TABLE 77. BUBBLE STABILITY AND STATIC ADSORPTION IN AQUEOUS SAPONIN SOLUTIONS

Saponin Concentration.	Average Duration of Bubbles.	Static Surface Tension.	Adsorption [5] $\Gamma = -\frac{c}{RT} \cdot \frac{d\sigma}{dc}$.
per cent.	seconds.	dynes/cm.	
0	0	72·8	0
0·00036	10	72·4	$0{\cdot}4 \times 10^{-10}$
0·00060	11	71·6	$0{\cdot}6 \times 10^{-10}$
0·0072	17	71·0	$0{\cdot}7 \times 10^{-10}$
0·0125	32	70·2	$1{\cdot}1 \times 10^{-10}$
0·0241	87	67·5	$1{\cdot}8 \times 10^{-10}$
0·0500	137	63·3	$2{\cdot}6 \times 10^{-10}$
0·100	149	58·1	$3{\cdot}0 \times 10^{-10}$
0·250	160	52·5	$3{\cdot}2 \times 10^{-10}$
0·500	171	—	$\Gamma\infty = 3{\cdot}3 \times 10^{-10}$
1·00	170	—	Saturation of the adsorption layer at C∞ = 0·1—0·15 per cent.

On plotting the above data with concentration of solution as abscissæ and bubble stability and $\Gamma \times 10^{10}$ as ordinates, it is found that the graphs are similar to the typical curve for the adsorption isotherm. The authors hold that bubble stability is enhanced by the presence of a stabiliser, *i.e.*, by adsorbed particles conferring rigidity on the surface. " Maximum static surface rigidity must be attained

[1] Kofler, *Chem.-Ztg.*, **48**, 165 (1924); Mand, *ibid.*, **50**, 850 (1926).
[2] Woodward and Alsberg, *J. Pharmacol.*, **16**, 237 (1920); Kofler, *Biochem. Z.*, **129**, 64 (1922); Wastl, *ibid.*, **146**, 376 (1924); Horn, *Am. J. Pharm.*, **98**, 53 (1926).
[3] Ostwald and Meissner, *Kolloidchem. Beihefte*, **26**, 17 (1928).
[4] *Kolloid-Z.*, **53**, 145 (1930).
[5] Calculated from the experimental σ—C graph.

when the surface is saturated, in other words, when the adsorption limit is attained." Thus, in the case of saponin, which can yield solid surface films, the stability of the bubbles increases steadily with the rise in concentration of the saponin to the limiting value set by adsorption. This is in marked contrast to the bubbles produced by a "liquid" adsorbed film such as for iso-amylal cohol, where *maximum* bubble stability is reached long before the adsorption limit is attained.

COLLOIDS AND THE SOLUBILITY OF GASES

The question of the influence of colloids on the solubility of CO_2 in water assumes considerable interest in the brewing industry. Creighton [1] and King [2] have summarised the available data in this connection.

Findlay [3] and his co-workers have carried out investigations on the influence of gelatin, glycogen, starch and other colloids on the solubility of various gases, provided adsorption and chemical reaction factors do not enter. Adsorption does occur above a definite pressure depending on the system under investigation.

The influence of colloids on gas solubility is very complex and specific effects must be recognised, depending on both the colloid, the gas, and the gas pressure.[4] In the case of dextrin, the solubility diminishes practically in exact proportionality with the increasing concentration of the colloid. Starch solutions required much greater time for saturation with the gas to become effected. Gelatin presented certain peculiarities, *e.g.*, dilute solutions are rapidly saturated with gas, but concentrated solutions more slowly, and on reducing the pressure the rapid gas evolution causes intense frothing. Again, CO_2 absorption appreciably lowered the gelatinising temperature. Egg albumin solutions exhibited a marked minimum solubility at 850 mm. Hg pressure. In view of the modern work on proteins, the study of gas solubility deserves renewed study, especially in relation to the pH value.

GAS CONTENT OF BEER

Earlier workers such as Langer and Schultze [5] taught that the solubility of CO_2 in beer was greater than in the corresponding alcohol-water liquid, and Prior [6] held this to be due to the presence of phosphates. However, the phosphate content of beer is too small

[1] Alexander's "Colloid Chemistry" (New York, 1926), Vol I., p. 634.
[2] *Brit. Assoc. Repts. Coll. Chem.*, **3**, 41 (1920).
[3] *Kolloid-Z.*, **3**, 169 (1908); *Biochem. J.*, **5**, 304 (1910); *J. Chem. Soc.*, **97**, 536 (1910); **101**, 1313, 1459 (1912); **103**, 636; 1170 (1913); **105**, 291 (1914); **107**, 282 (1915).
[4] Findlay and Creighton, *ibid.*, **97**, 536 (1910).
[5] *Z. ges. Brauw.*, **2**, 369 (1879); **6**, 329 (1883).
[6] *J. Soc. Chem. Ind.*, **18**, 288 (1899).

to influence CO_2 solubility. Similarly, Mohr's [1] contention of a chemical combination of CO_2 and alcohol is unsupported by experimental evidence.

Although Emslander and Freundlich [2] believed that the increased CO_2 solubility of beer was due to adsorption of the gas on the beer colloids, Müller [3] found that alcohol decreases the solubility of CO_2 in water, whilst Findlay and Shen [4] settled the issue by proving that CO_2 solubility was less in worts and beers than in water and independently of pressure. Accordingly, all the earlier work was vitiated owing to the solutions being saturated with CO_2.

The problem now becomes one of the rate of evolution of CO_2 from supersaturated solution. Findlay and King [5] investigated the effects of colloids and fine carbon particles on the rate of CO_2 evolution from well-agitated, supersaturated solutions and found that in water the rate of evolution closely parallels the degree of supersaturation, but in colloidal solutions wide variations occur, complicated by the previous history of the colloid, *e.g.*, thermal history, ageing, reversible or irreversible changes, *etc.*

Occasionally, colloidal solutions supersaturated with CO_2 can remain quiescent for 20 minutes or more, but such an unstable system immediately responds to slight mechanical shock with CO_2 evolution.[6] King [7] states that " the comparatively slow saturation of beer containing a high proportion of solids is, without doubt, to be interpreted as evidence of solution of the gas in the disperse phase, the rate of which is directly proportional to the rate of diffusion." The mechanism of such solution in the colloid phase is not known. However, assuming this to be the true explanation, it is obvious that carbonation of beer should be so conducted that CO_2 can dissolve in the beer colloids, *e.g.*, the beer should contain a high content of residual extract and be carbonated at low temperature.

HEAD ON BEER

A beer has a good " head " when the froth is not only voluminous but persistent. Not only must the colloid content of beer ensure a low surface tension, but the froth must be stabilised by finely-divided coagulated colloid material.

A considerable literature [8] has grown relating to the various

[1] *Wochsch. Brau.*, **21,** 363 (1904).
[2] *Z. physik. Chem.*, **49,** 317 (1904).
[3] *Wied. Ann.*, **37,** 24 (1889).
[4] *J. Chem. Soc.*, **101,** 1313 (1912).
[5] *Ibid.*, **103,** 170 (1913); **105,** 1297 (1914).
[6] Cf. Young, *J. Am. Chem. Soc.*, **33,** 48 (1911).
[7] *Loc. cit.* Cf. Langer, *Zeit. Brauw.*, **27,** 307 (1904).
[8] Fernbach, *Ann. Brass. Dist.*, **16,** 145 (1913); Fürnrohr, *Z. ges. Brauw.*, **36,** 473, 481 (1914); Rigaux, *Ann. inst. sup. fermentations Gand.*, **34,** 93 (1925); Raux, *Brasserie malterie*, **15,** 181 (1925); Kalk, *ibid.*, **15,** 117 (1925); *Wochsch. Brau.*, **42,** 85 (1925); Emslander, *ibid.*, **43,** 38 (1926); Baker and Fulton, *J. Inst. Brewing*, **29,** 747 (1923).

aspects of head on beer. Different colloids have been shown to play a *rôle*, *e.g.*, dextrin [1] and proteins, beer giving a better head when the action of proteolytic enzymes is reduced to a minimum, as by mashing at low temperatures.[2] The gas content is also an obvious factor and Ralk [3] observed that 0·375 per cent. of CO_2 gave a very good head on beer, whilst 0·300 per cent. produced poor results.

Various beer froths have been collected and analysed for comparison with the analysis of the original beers.[4] On the whole, the foam was richer than the original beer in total nitrogen, but less so in amide nitrogen. The foam contained more esters and total acid, whilst its content of volatile acid was from 2—10 times greater than in the original beer. The surface tension of the liquor produced by the collapsed foam was, as expected, less than that of the beer itself, and the Ramsden phenomenon was exhibited, numerous flecks of coagulated protein being apparent. Lüers and his co-workers emphasise that there is a mean optimum size [5] of colloid particles of nitrogenous matter for favouring foam formation, and, therefore, excessive proteolysis or too fine filtration should be avoided. Frequently, chilling will reduce foam tendency and froth retention because the colloid particles have coagulated and so passed the optimal size, but warming usually reverses this change.[6]

Windisch and Bermann [7] investigated the problem from a different angle. Wort was ultrafiltered with the Zsigmondy-Bachman filter [8] and the ultrafiltrate on being shaken gave a froth which was not persistent. The residue on the filter contained colloid matter, part of which had undergone irreversible coagulation. The ultrafiltrate regained its foam retention capacity when the soluble colloid matter was added to it from the filter. This reversible colloid matter proved to be mainly carbohydrate, together with some protein. The proteins which lead to froth formation are very surface-active, whilst the non-proteins, particularly barley gum, are not surface-active but act as foam stabilisers. The authors believe that there is an optimum ratio between the protein froth-former and non-protein froth-stabiliser. It was further observed that the addition of a very surface-active agent such as amyl alcohol or octyl alcohol entirely prevented the frothing of wort.[9]

The question of the influence of the size of the particles of beer colloids in relation to head has been studied by Geys [10] who finds foam formation and duration not to be due to the finest dispersed

[1] Ihnen, *J. Soc. Chem. Ind.*, **24**, 504 (1905).
[2] Fürnrohr, *loc. cit.*
[3] *Wochsch. Brau.*, **42**, 76 (1925).
[4] Lüers, Gey and Braumann, *Z. ges. Brauw.*, **43**, 185, 193, 201 (1920).
[5] Cf. Petit, *Brasserie malterie*, **16**, 49, 65, 81 (1926).
[6] Petit, *ibid.*, **6**, 161 (1916).
[7] *Wochsch. Brau.*, **37**, 109, 121, 129, 137, 145, 153 (1920).
[8] *Z. anorg. allgem. Chem.*, **103**, 119 (1918).
[9] Cf. Rehbinder and Wenström, *Kolloid Z.*, **53**, 145 (1930).
[10] *Wochsch. Brau.*, **44**, 145 (1927).

particles whose electric charge is unfavourable to their coagulation, but to the coarser particles of nearly neutral electric charge. It is these particles which become adsorbed at the air/liquid interface. Coarsening of the dispersion of the colloids is progressive during the later stages of brewing from the cooling of the wort onwards. Excessive coagulation of the colloid must be avoided since this would result in the removal of head-forming material. Again, long contact of beer with yeast is unfavourable to a good head, the yeast enzymes peptising the colloids and leading to a finer degree of dispersion.

Geys [1] has also dealt with pH influences.[2] Fermentation at low temperatures leads to a low concentration of hydrogen ions. Particularly with greater quantities of yeast, there is a tendency, especially towards the end of the fermentation, for the stage of increasing or constant acidity to revert to a more alkaline reaction. Now, as the pH value falls, there is a coagulation of proteins, a condition favourable to a good head. In the opposite direction, peptisation of the ultramicrons of the beer would lead to poor frothing qualities since there is much less tendency for the peptised colloids to be adsorbed at the air/liquid boundary, and the finer dispersion of the colloids is unfavourable to the presence of the nuclei which should assist CO_2 evolution.

In the boiling of wort with hops, the very surface-active hop bitter acids [3] are introduced. These have been investigated by Lüers and Baumann [4] who, by determining surface tension and making ultramicroscopic examination and ultrafiltration, found that humulone [5] (α-bitter acid) is intermediate between suspensoid and emulsoid colloids in character. The degree of dispersion of humulone is a function of the presence of electrolytes and other colloids.

Humulone lowers the surface tension of water to a greater extent in the acid range. It is adsorbed at the air/liquid interface and permits froth formation, but dextrin and the colloid products of protein hydrolysis are necessary for stabilisation of the froth. During boiling of the hops hydrolysis [6] of humulone occurs with the consequent diminished power to reduce the surface tension of water. This effect is the greater the longer the boiling and the greater the acidity, but is not so marked when humulone is boiled with wort, since the wort protein exercises a protective action. The other hop constituents, lupulone (β-bitter acid) and γ-resin do not possess such

[1] *Ibid.*, **43**, 439 (1926). See the work of Windisch *et al.*, *ibid.*, **42**, 239, 251 (1925), with similar conclusions. Lüers and Schmal, *Brasserie malterie*, **16**, 90 (1926), find most persisting foams with worts at pH 6—6·9.

[2] Head formation is independent of pH in beers from unhopped worts ; Windisch *et al.*, *Wochsch. Brau.*, **43**, 207 (1926).

[3] Cf. Lintner, *Z. ges. Brauw.*, **14**, 357 (1891) ; **27**, 668 (1904) ; *Z. Nahr. Genussm.*, **18**, 252 (1909) ; *Ber.*, **31**, 2023 (1898) ; Wöllmer, *ibid.*, **49**, 780 (1916).

[4] *Kolloid-Z.*, **26**, 202 (1920).

[5] Cf. Wöllmer, *Wochsch. Brau.*, **42**, 1 (1925) ; Wieland *et al.*, *Ber.*, **58**, 102 (1925).

[6] Wöllmer, *Z. ges. Brauw.*, **40**, 372 (1917) ; Windisch *et al.*, *Wochsch. Brau.*, **41**, 281 (1924).

pronounced surface activity. Humulone has from 2·5—5 times the foaming power of lupulone [1] and all the foaming material in hops is extractable by ether.[2]

COLLOID CHEMISTRY OF HOPS

The investigation of Lüers and Baumann may now be mentioned in greater detail as several interesting results were noted. Both the hop bitter acids lower the surface tension of water. In low concentration (0·1 millimol/litre) they yield sols almost clear, but at higher concentrations (0·3—0·4 millimol/litre) the sols are opalescent or milky. The stronger sols are prepared by adding an alcoholic solution to water. Such a 0·3 millimol/litre sol was ultrafiltered and the surface tension of the ultrafiltrate determined as 68·57 dynes/cm. The surface tension prior to ultrafiltration was 56·54 dynes/cm. whilst the surface tension of the corresponding alcohol-water mixture was 69·5 dynes/cm.

The freshly-prepared opalescent sol rapidly develops turbidity and the degree of dispersion is such that the Tyndall cone is resolvable with a magnifying glass. There is a corresponding rise in the surface tension, *e.g.* a sol with surface tension of 51·53 dynes/cm. when fresh, showed a surface tension of 58·91 dynes/cm. after standing for 120 minutes.

Electrolytes render the dispersion coarser and raise the surface tension, there being exhibited a lyotropic anion influence : $Cl < CNS < I < SO_4 <$ tartrate. Rochelle salt, however, in small amount, causes the sol to become clear and the ultramicroscope then shows a great increase in the number of particles possessing Brownian motion. The effect whereby Rochelle salt increases the dispersion of humulone is not due to a complex compound being formed, but is physico-chemical, the electric charge being raised by ion adsorption.

The degree of dispersion of humulone is scarcely affected by starch or dextrin but is very noticeably raised by gum arabic and gelatin, which act as protective colloids in the system. The influence of pH was investigated in the case of a 0·3 millimol/litre sol of humulone, using phosphate buffers. For pH values of 4·49, 5·88, 6·55, 9·1, the surface tension determinations were 56·34, 56·60, 58·97 and 60·42 dynes/cm. respectively, whilst the corresponding appearances of the sol were : very turbid, opalescent, clear and clear respectively. Phenolic salts are formed, less surface-active than humulone and having a very bitter flavour.

Humulone is adsorbed by animal charcoal in accordance with the adsorption isotherm, but Rochelle salt diminishes such adsorp-

[1] Windisch, *ibid.*, **43,** 207, 217, 229, 241, 253 (1926).

[2] For detailed analyses and a physico-chemical study of hops reference should be made to the work of Huyghe and van dem Eynde, *Bull. assoc. élèves inst. sup. fermentations Gand.*, **29,** 348 (1927).

tion. The less the degree of dispersion of the sol, within the colloidal range, the more pronounced is the adsorption on charcoal.

When wort is boiled with hops, the coagulated protein adsorbs humulone. Again, wort-humulone sols are clearer than the usual aqueous sol, since the wort proteins act as protective colloids. Although the simple hydrosol will froth on agitation the froth is not stable, but stability is gained by the presence of wort proteins or by gelatin. Analysis showed the concentration of gelatin in the froth. Humulone, too, concentrates in the froth, as its marked bitterness indicates.

When humulone is boiled in water, oxidation occurs and the change in the humulone is reflected in the much higher surface tension, increased solubility and more bitter taste. Such change is more marked in an acid medium than in an alkaline medium, and also occurs on boiling in wort but to a less degree owing to the protective action of the proteins.[1]

COLLOID CONTENT OF BEER: FINING

The colloid content of beer has been investigated by electrodialysis [2] and by ultrafiltration,[3] and the problem bears definite relation to the palatability of beer.[4] A method developed by Marc [5] is to add an insoluble crystalline powder [6] to the beer to adsorb colloid matter and then determine the solute remaining by means of the Zeiss interferometer.

Before beer in casks is sent out to the trade a small quantity of a " fining " agent is often added, in order to accelerate clarification. Thus, Irish moss [7] and isinglass [8] are in common use. The optimum fining of beer is the net result of the coagulation of beer colloids by the addition of a colloid of opposite electric charge and by the influence of ions of opposite charge. Excess of a fining agent, *e.g.*, isinglass, can result in stabilising haze colloids, when it becomes necessary to add a further colloid of opposite electric charge. As example, Irish moss will mutually precipitate isinglass.

COLLOID PROBLEMS OF WINES

Colloid problems are commonly encountered in the preparation of wine [9] notably in regard to its clarification,[10] and in the phenomenon of " casse " or dregs in bottled wine.

[1] See Windisch, Kolbach and Wentzell, *Wochsch. Brau.*, **42**, 287, 295, 303, 313, 323, 333 (1925) for a study of changes in degree of dispersion of proteins in the various stages of brewing.

[2] Windisch, Kolbach and Borges, *ibid.*, **47**, 67, 77 (1930).

[3] Windisch and Dietrich, *ibid.*, **37**, 231, 237 (1920).

[4] Mendel, *Allg. Z. Bierbrau, Malzfabr.*, **45**, 50 (1909).

[5] *Kolloid-Z.*, **14**, 181 (1914).

[6] Marc and Sack, *Kolloidchem. Beihefte*, **5**, 375 (1914).

[7] Ranken, *J. Inst. Brewing*, **35**, 287 (1929).

[8] Harman, Oliver and Woodhouse, *ibid.*, **34**, 203 (1928).

[9] For the modern method of manufacture see De Castella, *J. Dept. Agr. Victoria*, **27**, 651 (1929).

[10] Astruc, *Prog. agr. vit.*, **92**, 60 (1929); Brown, *J. Inst. Brewing*, **20**, 345 (1914);

The details of wine manufacture[1] are beyond the scope of this volume. Very briefly, grapes are crushed without crushing the seeds and the aerated "must" is fermented. In making white wines the skins are separated after crushing in order to avoid the extraction of colouring matter, and *debris* is removed by addition of egg albumin. This mutually flocculates the tannin in the must and the bulky precipitate sweeps down all impurities. The tannin content of the must is previously fortified by adding 2—8 gm. of tannin per hectolitre.

Fermentation completed, the wine is clarified by colloid treatment, centrifuging proving of no avail.[2] Siliceous earth,[3] charcoal, filter-paper and Spanish clay are frequently employed. Clarification or "fining" is, however, most commonly conducted by the mutual flocculation of colloids, *i.e.*, the tannin in the wine and an added colloid such as milk, casein, egg-white, gelatin[4] or isinglass. Removal of tannin may improve the flavour of astringent red wines, but if tannin initially be present in too small a quantity as in some white wines, it must be previously added, the usual amount being 20—25 gm. per cask.[5]

THE FINING OF WINE

That clarification is just a mutual colloid flocculation with its attendant mechanical aid in removing impurities is to state the problem very simply. The actual colloid-chemical phenomena involved deserve study, and an interesting investigation in this connection has been made by Rüdiger and Mayr.[6] They considered the important case of wine clarified by the use of gelatin.

Different gelatins proved to possess different efficiencies in flocculation, thus explaining the contradictory results of various workers. The actual turbidity of the wine is an important factor in assisting clarification. It was found that the longer the gelatin was soaked in water prior to making into solution, the more effective was its fining power. The water used for soaking should be that employed in the solution of the gelatin.

The concentration influence of the gelatin solution is a function of the age of the solution. With increasing age, 0·5 and 1 per cent. solutions showed a marked falling off in efficiency, this running parallel with the increase in viscosity of the sols, the formation of

Malvezin, *Bull. soc. chim.*, **47,** 1194 (1930); Manceau, *Ann. fals.*, **20,** 7 (1927); Mathieu, *Chimie et industrie*, Special No. **608** (Sept., 1925); Rüdiger and Mayr, *Z. angew. Chem.*, **41,** 809 (1928).

[1] See Hewitt, "The Chemistry of Wine Making: a Report on Œnological Research" (London, 1928); Fabre, "Traité Encyclopédique des Vins," Tome I. (Algiers, 1929).

[2] Astruc, *Prog. agr. vit.*, **92,** 60 (1929); Dubaquié, *Ann. fals.*, **21,** 460 (1928).

[3] See the detailed studies by Caldwell, U.S. Dept. Agr., *Bull.*, **1025** (1922).

[4] Rüdiger and Mayr, *Kolloid-Z.*, **46,** 81 (1928).

[5] Carles, *Ann. chim. anal.*, **18,** 440 (1914); Levallois, *Ann. fals.*, **19,** 15 (1926); Moreau and Vinet, *Rev. vit.*, **41,** 457 (1914); *Bull. soc. agr. France* (1919), p. 16.

[6] *Kolloid-Z.*, **47,** 141 (1929).

coarser gelatin aggregates and the decrease in adsorbing surface thus available. Heating the sols to 80° C. regenerates the fining capacity due to the breaking down of the aggregates. The 0·25 per cent. solution, on the other hand, becomes more effective as it is aged, until an optimum is reached after 2—3 days. The authors attribute this striking phenomenon not to a change in the degree of dispersion of the gelatin but to some unexplored changes in the electric charge of the aggregates.

Clarification with gelatin alters the chemical analysis of wine, the tannin content falling and the nitrogen increasing (due to gelatin remaining in solution), whilst the viscosity decreases and the surface tension is raised. The pH value is unchanged.

Within certain limits, an increase in the acidity of the system

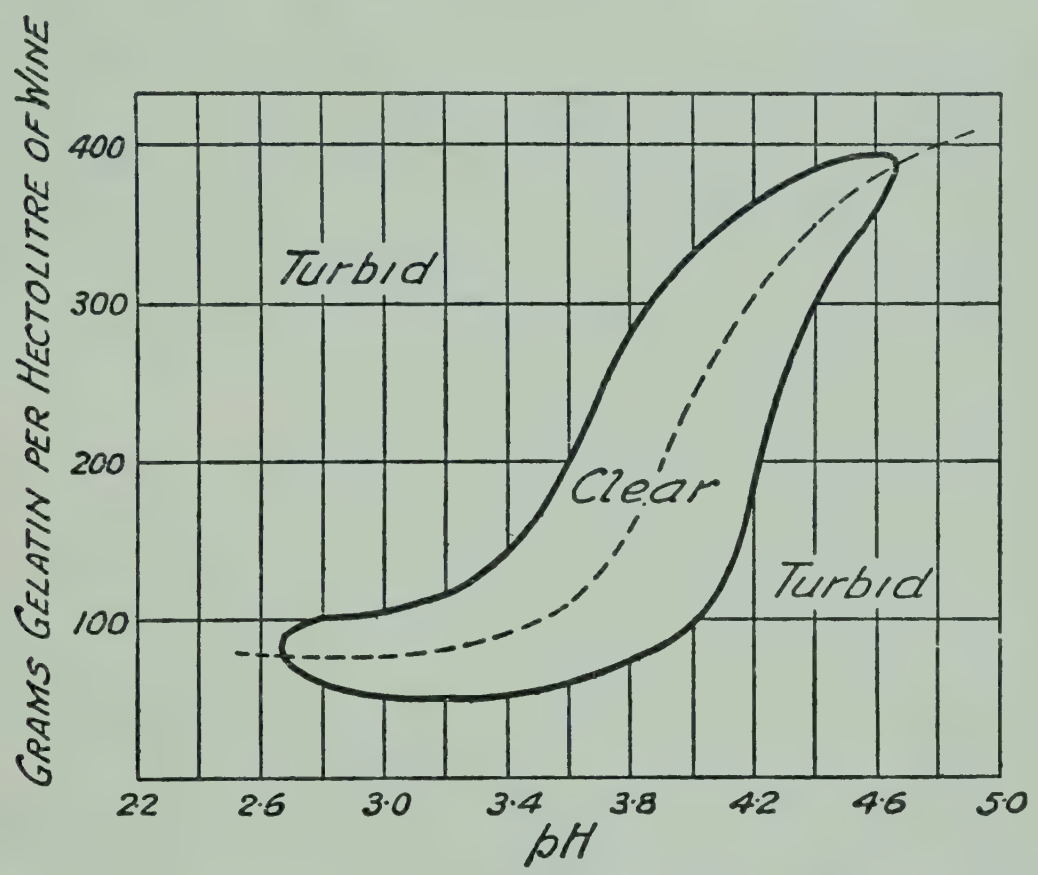


Fig. 56.—The clarification of a wine by gelatin in relation to pH value.

favours clarification and this explains the technical advantage accorded the use of isinglass as against gelatin, since the former is employed in acid solution. In an example quoted by the authors, decreasing the pH value from 4·2 to 3·4 resulted in only 140 gm. of gelatin being necessary to fine 1 hectolitre of wine, as against 240 gr. The nature of the acid used is without influence on these results.

Gelatin may, or may not, bring about clarification, according to its concentration. There is thus a clarification zone. Cataphoresis tests showed that the haze particles in wine are negatively charged. Since the pH value of the wines employed was about 3·9, the gelatin would carry a positive charge. Flocculation occurs in a narrow zone and when neutralisation of the charges on the gelatin and haze particles is complete, there is a marked precipitation optimum and a maximum coagulation velocity. If the discharge of electric sign is

incomplete, but the potential of the resulting secondary particles is below the critical potential, flocculation is gradual but eventually complete. With considerable excess of gelatin, or of haze, the secondary particles assume the sign of the excess colloid and haze persists. The authors confirmed this by cataphoresis.

Since agar sols are negatively charged, even in the presence of excess acid, a wine hazy with excess gelatin could be clarified by addition of agar, as could also certain special wines where a persistent haze due to bacterial contamination was found to be positively charged.

The pH influence is now explicable. Gelatin is more positively charged the more the pH value is below the isoelectric point, pH = 4·7. Also, the negative charge on the haze particles of the wine is reduced as the acidity increases towards their isoelectric point, which is still further in the acid field. Both effects are additive to the end that a smaller gelatin addition is required on the acid side.

Fig. 56 reproduced from Rüdiger and Mayr's paper, shows a clarification-diagram relating to the clarification of a pear wine by gelatin. The dotted line corresponds to the optimum gelatin concentration giving most rapid flocculation at the pH value in question.

THE CASSE OF WINE

The " breakage " or " casse " of wine is the phenomenon shown when a bright wine after drawing from the cask becomes turbid and throws down a precipitate, or when the wine in an opened bottle " films." Two forms of casse are recognised : white casse and blue casse.

Laborde [1] found white casse to be colloidal ferric phosphate. Low acidity produces a black casse, which is transformed by tartaric or sulphurous acid into the white. The ferric phosphate carries down proteins, tannin and calcium.[2] The use of ammonium phosphate (to increase fermentation) [3] or poorly-washed charcoal (in the clarification of the wine) accentuates the trouble. Citric acid, 0·5—1 gr. per litre, will prevent white casse. Tartaric and malic acids, but not lactic or succinic, will serve the same purpose, due to the solubility of the phosphate in the acid.[4] Oxidation (by aeration) followed by gelatin will remove the casse after formation.[5]

Farbre and Brèmond [6] have shown that wine prepared from grapes pressed by treading and then fermented and matured in

[1] *Compt. rend.*, **164,** 441 (1917). See also Moreau and Vinet, *Bull. soc. agr. France* (1917), 267 and 292.

[2] Fonzes-Diacon, *Compt. rend.*, **164,** 199, 650 (1917).

[3] Moreau and Vinet, *Rev. vit.*, **41,** 679 (1914).

[4] Weil, *Z. Untersuch. Lebensm.*, **29,** 60 (1915). Passerini, *Staz. sper. agrar. ital.* **39,** 241 (1906) describes a ferric lactate casse.

[5] Baragiola, *Z. Untersuch. Lebensm.*, **33,** 513 (1917).

[6] *Ann. fals.*, **20,** 213 (1927).

wooden or glass vessels contains 10—14 mg. per litre of ferrous salts. The use of ferroconcrete vats increases the iron content about 5 times and any addition of SO_2 during fermentation results in considerable iron due to corrosion. Again, aluminium dissolved from glass may yield a white casse due to its precipitating action on the pectin in grape juice.[1]

Brown [2] carried out considerable research on the white casse of South African wines. He found that ferrous iron functions as a catalyst in the oxidation [3] of various ingredients in the wine, the casse being a colloidal complex of iron, tannin and proteins.

The removal of iron from wine by means of potassium ferrocyanide is now occasionally practised, but an overdose of this reagent results in a greenish opalescence.[4]

The blue casse is usually associated with rich and highly-coloured wines. Marsais [5] has described the light blue, opalescent suspension in champagne wines and advises care against aeration of the filtered wine. Blue casse again involves iron salts and is a complex with tannin and the colouring matter of the wine.[6] It is most intense in very acid wines.[7] Some workers believe an oxidase is involved.[8]

Unfortunately the whole problem of casse seems to have been treated somewhat empirically and no systematic investigation has been made into the colloid content and colloid stability of wines. The present writer feels that adsorption at the air-liquid interface, with consequent " denaturation " of a protein complex, may be a factor.

[1] Dubaquié, *ibid.*, **18,** 418 (1925) ; **19,** 459 (1926).
[2] *J. Inst. Brewing,* **20,** 345 (1914).
[3] See Lo Priore, *Staz. sper. agrar. ital.*, **50,** 382, 394 (1917).
[4] Reichard, *Z. Untersuch. Lebensm.*, **53,** 163 (1927).
[5] *Rev. vit.*, **72,** 283 (1930).
[6] Piedallu *et al.*, *Compt. rend.*, **170,** 1129 (1920).
[7] Fonzes-Diacon, *Compt. rend. agr. France,* **11,** 436 (1925) ; Ventre, *ibid.*, **11,** 466 (1925).
[8] Wolff and Grandchamp, *Compt. rend.*, **181,** 939 (1925) and French Pat. 613,922 (1925).

CHAPTER XII

THE FREEZING AND THAWING OF COLLOID SYSTEMS

The refrigeration of foodstuffs as a means of preservation, particularly in transport or prolonged storage, is of prime importance in a national food supply, whilst damage to fruits, cereals and vegetables by frost also focuses attention upon the changes incidental to the freezing and thawing of foodstuffs in general. As a whole, the subject involves consideration of the effects of freezing and thawing on colloid systems, particularly hydrophile systems.

In the case of the simpler hydrophobe systems,[1] the issue is more clear. The hydrosol in freezing becomes more concentrated in colloid and electrolytes, so that flocculation of the colloid may ensue, depending on the original concentration of electrolyte. If only incipient coagulation takes place, the colloid particles remain distributed between the ice crystals as they form. The packing of the colloid particles is always more close than in the original sol. For coagulation to be permanent, the time factor enters in, the sol having to remain frozen long enough to permit aggregation of the colloid particles. Again, the greater the degree of peptisation of the colloid, the more readily will be the repeptisation of the frozen flocculated colloid on thawing. The shorter the time the system remains frozen, the greater is the chance that thawing will prove entirely reversible.

Foodstuffs, however, are more complex systems, consisting of proteins and/or carbohydrates in an aqueous medium, with, in some cases, the additional complication of ingredients in emulsion or suspension form. The fundamental problem throughout remains the same, *viz.*, the degree of reversibility to the original state on thawing the frozen food. Modern developments in this field relate especially to meat, fish and eggs, and an extensive literature is accumulating. Research work of the highest importance is in progress at Cambridge under the direction of Sir W. B. Hardy and to this work our main attention will subsequently be directed.

Bottazzi and Bergami [2] have investigated the effect of low temperatures on protein solutions and observed that at 0° C. coagulation only ensues if the electrolytes are first removed by dialysis. The coagulation is, however, reversible in most cases.

[1] Freundlich, " Colloid and Capillary Chemistry " (London, 1926), p. 475.
[2] *Arch. sci. biol.* (*Italy*), **6,** 74 (1924).

Neutral electrolytes effected protection of dialysed proteins against low temperature coagulation. In the isoelectric region the coagulation tendency is enhanced,[1] *e.g.*, in the case of ox blood serum, although ordinarily it is reversible after being maintained at — 25° C.

FREEZING AND THAWING

A detailed review of the physics and chemistry of freezing and thawing in relation to foods has been given by Stiles [2] and the student of this subject should certainly consult his monograph. The cooling of a small body throughout a temperature range $T_1 \rightarrow T_2$ will, in the absence of change in phase, result in a continuous temperature drop, particularly if the thermal conductivity is great. The nearer the cooled body is in temperature to the cooling medium, the slower is the course of the cooling. Freezing complicates the situation, for during freezing, the time-temperature curve will remain almost constant (parallel to the time axis).

The time taken in cooling is a function of (*a*) internal factors characteristic of the cooled body, *e.g.*, thermal conductivity, specific heat, density, latent heat, and the extent and nature of the surface, and (*b*) external factors characteristic of the chilling medium, *e.g.*, temperature, thermal conductivity, specific heat, density, and degree of agitation. Stiles discusses all these in considerable detail.

It is important to reflect that ice possesses different values for thermal conductivity and specific heat than water.[3] Quite approximately, the thermal conductivity of ice is four times that of water, whereas its specific heat is half that of water. Accordingly, tissues of 80 per cent. water content, will when *frozen* cool 6·4 times as quickly as when not frozen, complicating ingredients being absent. The practical consequence of this is that when a foodstuff is frozen throughout its mass, its temperature drops rapidly to that of the chilling medium. Again, the time necessary to thaw the frozen food will considerably exceed that required in the freezing process.

The main factors controlling the rate of cooling are, of course, the temperature and the thermal conductivity of the external medium effecting cooling.

Since water is present in meat, fish, fruit and vegetables to about 80 per cent. by weight, Stiles deals with the simple case of the freezing of water. At one atmosphere pressure water and ice are in equilibrium at 0° C ; each increase in pressure of one atmosphere lowers the freezing point by 0·007° C. However, the phenomenon of supercooling is sometimes met, where water remains as liquid below

[1] See Bergami, *Atti, accad. Lincei.*, **6,** 117 (1927), for a similar observation regarding the crystalline lenses of sheep and of cattle which become opaque at 0° C. more readily and to a greater degree when immersed in Ringer solution at pH 4·2, the isoelectric point.

[2] Food Investign. Board, *Special Rept.*, No. **7,** 1—86 (London, 1922).

[3] See Barnes and Maas, *Can. J. Research*, **3,** 205 (1930).

0° C. even under atmospheric pressure. The possibility of supercooling is greater in the absence of air and in capillaries, but its incidence is instantly checked by adding a trace of the solid or by shaking the liquid. Extensive investigations on this subject were made by Tammann, who found that in order for a crystalline solid to solidify from supercooled solution, a number of crystallisation centres must be present. The velocity of crystallisation is another important factor. Slow cooling of a liquid to just below its freezing point leads to few crystallisation centres, but high crystallisation velocity, so that a very coarse-grained solid results made up of a few large crystals. Still further below the freezing point, both the crystallisation velocity and the number of crystallisation centres are high and the resulting fine-grained solid is a mass of small crystals. Rapid cooling may lead to an amorphous condition of the solid, since both the crystallisation velocity and the number of crystallisation centres are very small.

Coming now to aqueous solutions, and taking NaCl as a typical solute, we find that freezing results in ice separation and in the corresponding increasing concentration of the solution, until that point is reached when both ice and NaCl began to separate. At this stage, the entire solution will become solid, and the temperature at which this phenomenon occurs is known as the *eutectic point*. This temperature does not change again until solidification is complete, when further fall in temperature accompanies increased cooling.

Thawing is of equal importance to freezing, and its rate affects the subsequent condition of the foodstuff. Stiles' detailed discussion should be read in this connection. When freezing is so rapid as to fix the original spatial distribution of the colloid, thawing may be either rapid or slow and yet reproduce the original structure of the food in question. With slow freezing, however, resulting in large ice crystals, rapid thawing cannot restore the original condition. Again, if the colloid does not produce a sol or gel with simple contact with water, the rate of thawing is without interest subsequent to slow freezing. But, if the sol or gel condition follows contact of the colloid with water, thawing of the frozen system should be slow enough to allow the colloid to take up the water provided during melting. Rapid thawing under these conditions might result in a more or less permanent separation of the colloid and water.

THE FREEZING OF MILK

Milk freezes [1] at — 0·54° C. to — 0·57° C. (see p. 203). Parker and Spackman [2] have shown that the acidity of the milk plays a part in defining the freezing point and that a correction is necessary,

[1] Van Raalte, *Analyst*, **54**, 267 (1929); Andrew, *ibid.*, **54**, 210 (1929); Elsdon and Stubbs, *ibid.*, **55**, 423 (1930); Krenn, *Milchwirtschaft. Forsch.*, **7**, 436 (1929); Doan, *J. Dairy Sci.*, **10**, 353 (1927).

[2] *Analyst*, **54**, 217 (1929).

viz., 0·003° C. for each 0·01 per cent. excess acidity over the normal value of 0·14 per cent. This correction holds between acidities of 0·17—0·60 per cent. ; the correction for acidity values ranging from 0·14—0·17 per cent. is given as 0·010° C. Pasteurisation at 63° C. for 30 minutes raises the freezing point by 0·01° C.

Treating milk as an aqueous solution of lactose and salts, the fat and proteins in colloidal suspension and without influence on the freezing point, freezing will cause a separation of pure ice (freezing point). Further freezing brings about further separation of ice and a continuous concentration of solutes in the liquid phase. In a still liquor such concentrated solution is forced to the centre and the fat rises. Thus, Corblin [1] froze milk for 48 hours at — 10° C. Analysis of the upper layers showed excess fat, whilst the middle and bottom layers were rich in lactose and casein. On thawing, the cream floated *as globules* on the surface. He advised the freezing of milk in thin layers at — 15° C. with constant stirring in order to gain an homogeneous mass which would melt to normal milk again.

Although freezing in air and subsequent thawing causes irreversible changes in milk, freezing in liquid air will permit subsequent total reversibility. Frozen milk is a flaky mass (schistose condition), the ice crystals being enveloped with protein. According to Reid,[2] the freezing of milk causes calcium caseinate to be thrown out in particles visible [3] to the eye at low temperatures. The fat emulsion is never completely restored on thawing and the fat content of the upper layers may reach as great as three times that of the control sample. The fat content is greater also in the centre of the upper layer than in the periphery.

Reid observed that milk which has been frozen and then thawed showed a decrease in the length of the cream column. Due to changes in the emulsifying agent present, *e.g.*, removal of casein, there is clumping of the fat globules and creaming is accentuated. On thawing frozen milk, the serum is seen to be bluish and watery. The cream at first seems coarse and granular, an appearance which gradually passes, until in about 20 hours the original smooth condition is apparent.

The general problem of the effect of freezing on prepared emulsions remains open to investigation,[4] particularly in relation to the nature of the emulsifying agent and the size-frequency analysis of the initial system.

The freezing points of cream and skim milk are no different from that of the milk from which they were separated.[5] This is to be

[1] *Rev. gén. Froid.*, **9,** 113 (1928) ; *Bull. soc. encour. ind. natl.*, (1928), 232.
[2] Univ. Missouri Agr. Expt. Sta., *Res. Bull.*, **100** (1927).
[3] Cf. Fuld and Wohlgemuth, *Biochem. Z.*, **5,** 118 (1907).
[4] Certain preliminary experiments are mentioned by Vickery, " Report of the Food Investign. Board for Year 1929 " (London, 1930), p. 24.
[5] Doan, *J. Dairy Sci.*, **10,** 353 (1927).

expected since only the milk serum with its true solutes comes into question.

FREEZING OF CHEESE

The freezing point of cheese varies with the kind of cheese, its moisture content and its NaCl content. Watson and Leighton [1] have determined the freezing point of nine cheeses, obtaining values of — 1·2° C. for Cottage cheese to — 16·3° C. for Roquefort cheese, the respective moisture contents being 78·7 per cent. and 39·2 per cent. Leighton [2] points out the need for investigation of the decrease in freezing point of cheese on curing, water probably combining with protein and being removed from its *rôle* as solvent.

Sommer [3] has done research on this problem in the case of Cheddar cheese. He obtained freezing points ranging from — 4·3° C. to —14·3° C., the lowest value being for cheese over a year old. The age or ripeness of the cheese is a factor inasmuch as during ripening there is a marked increase in water-soluble substances.

The injury of cheese by freezing was studied by subjecting cheeses to temperatures well below their freezing points for different times and then thawing gradually. "Freezing injury is confined primarily to the texture, which becomes more or less crumbly." The injury to the texture is particularly evident at the sutures where the curd particles coalesced during the pressing process. However, in those samples where the sutures were well knit and mechanical holes were practically absent, texture injury was a minimum. It is interesting to note that storing the frozen cheeses for about one month at normal cheese storing temperature brought about so marked an improvement in texture as to render it practically impossible to distinguish cheese which had been frozen from the control samples.

FREEZING OF GELATIN GELS

Necessary to the more complicated study of the colloidal systems presented by such foods as meat and fish, is the detailed investigation of a typical colloidal system involving sol $\rightleftharpoons$ gel conditions. Aqueous solutions of gelatin meet this condition and most important work has been carried out thereon at Cambridge.

Moran [4] employed isoelectric gelatin of ash content 0·05 per cent., made into discs of gel with diameter 1·5 cm. and thickness 0·3 cm. He investigated the behaviour of gels on freezing and thawing from the points of view of (1) the micro-structure resulting from freezing, (2) quantitative estimation of the ice separating out, (3) changes in volume.

[1] *Ibid.*, **10,** 331 (1927).
[2] "Fundamentals of Dairy Science" (New York, 1928), p. 226.
[3] *J. Dairy Sci.*, **11,** 9 (1928).
[4] *Proc. Roy. Soc.*, **A 112,** 30 (1926). *Vide* also Kinoshita, *Bull. Chem. Soc. Japan*, **5,** 261 (1930).



FIG. 57.—Twelve per cent. gelatin gel frozen very rapidly in liquid air. Note small masses of ice about 3μ in diameter. (Mag. $\times$ 125.)

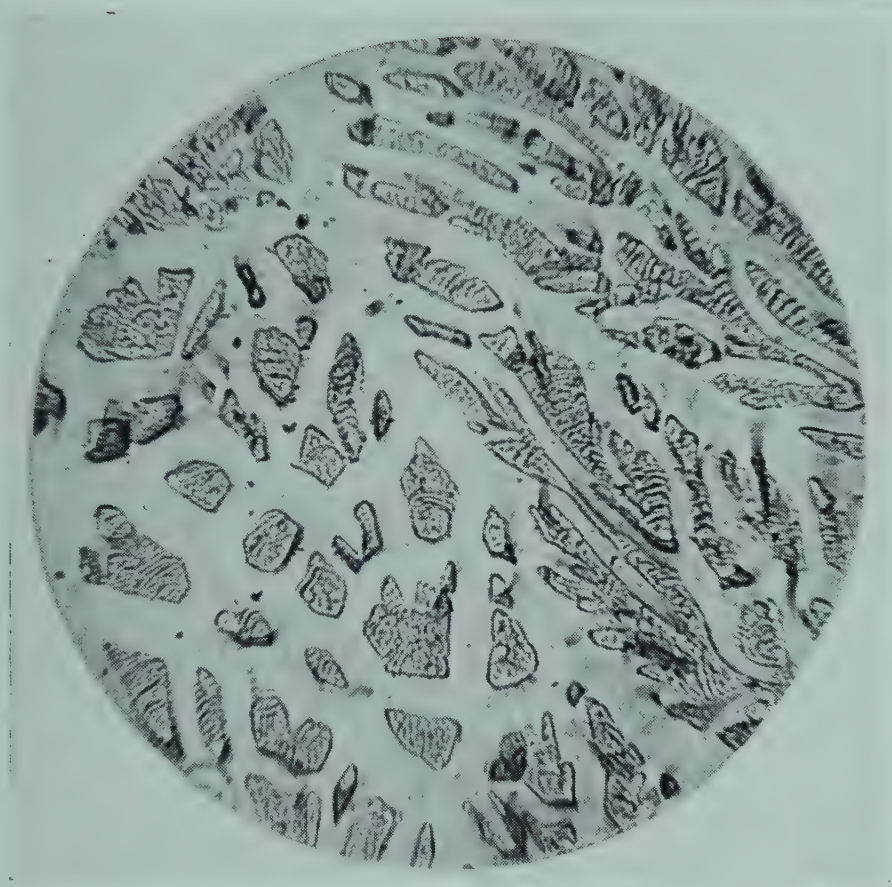


FIG. 58.—Twelve per cent. gelatin gel frozen slowly in air at 11° C. Note centres of solid solution of ice and gelatin (Hardy). (Mag. $\times$ 40.)

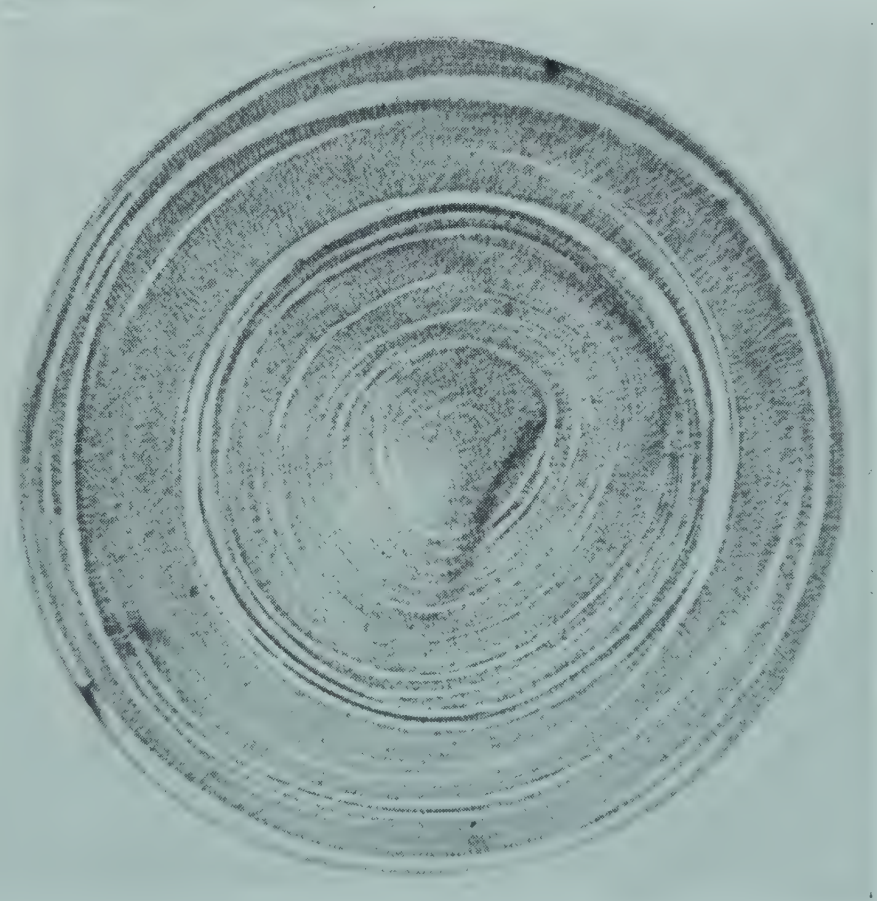


FIG. 59.—Liesegang ring formation of gelatin and solid solution (gelatin—ice) in concentrated gels frozen at medium freezing temperatures. (Mag. $\times$ 30.)

To face p. 410.]

Freezing of gelatin gels causes ice separation, the residual gel being relatively dehydrated and the micro-structure dependent on the rate of freezing. On thawing, the reabsorption of water may be difficult. A qualitative study was made of the micro-structure in relation to the gel concentration and the temperature of the freezing operation. (See Figs. 57, 58 and 59.)

Gels of 12 per cent. Concentration. Freezing in liquid air results in the interior of the gel becoming occupied by numerous clear spherical spaces of about 3 μ diameter and arranged in rows. These spaces correspond to actual centres of crystallisation and ice was not detected on the surface of the gel. When, however, the latter is frozen in air at —11° C., the interior contains irregular spaces holding spongy gel with homogeneous gel separating these spaces. Freezing commences at relatively few centres in the interior and also on the external surface of the gel. Finally, in the case of a gel frozen in air at — 3° C., the interior is free from spaces and freezing is confined to the external surface, a thick envelope of ice forming and enclosing a core of dehydrated gel which was completely reversible on thawing. Moran finds, therefore, two groups of possible centres of crystallisation, external and internal. The external centres are prepotent, they alone functioning in slow freezing.

Gels of <12 per cent. Concentration. In these gels, freezing at —3° C. indicated some active internal centres of crystallisation, whence it is deduced that weaker gels demand a slower rate of cooling in order entirely to suppress internal centres of crystallisation.

Gels of 38 per cent. Concentration. On freezing in air at — 3° C. ice is formed only externally. When freezing is more rapid, at — 11° C., a new phenomenon appears. The irregular spaces containing spongy gel (observable in 12 per cent. gels) are now replaced by spheres, each consisting of an ordered alternation of shells of ice and gel concentric to the original centre of crystallisation. If freezing is first conducted at —11° C., until the initial appearance of these spheres and the gel is then completely frozen at — 3° C., the concentric ring structure does not form, continuous ice formation taking its place.

Moran now studied the phase equilibrium between ice and gel. Using isoelectric gelatin and gels in the concentration range 12—40 per cent., he found that slow freezing (in air at — 3° C.) causes water to pass into the external shell of ice until the gel reaches a constant concentration, *viz.*, 54·3 per cent. gelatin. The equilibrium gel concentrations corresponding to the freezing temperatures of — 3°, — 5°, — 7°, — 11°, and — 19° C. respectively were found to be 54·3, 60·1, 62·1, 64·4 and 65·2 per cent. gelatin respectively, The results are the same no matter from which side the particular freezing temperature is approached.

On immersing a 65·5 per cent. gel in liquid air, Moran found no ice formation. The gel remained clear and transparent, whereas all gels containing less gelatin would become white and opaque. Hence, 35—34 per cent. water in a gelatin gel means no ice separation at any temperature, and this fact introduces the conception that water exists in such gels in two states: "bound" water and "interstitial" water.

THE PROBLEM OF BOUND WATER

Bound water cannot be frozen and is presumably in union with gelatin molecules, the spaces between acting as capillaries. In the present state of knowledge Moran believes that "the most satisfactory concept is to figure the gelatin molecule or aggregate being surrounded, first, by an envelope of combined water, and then by successive layers of non-combined water up to a critical distance, depending upon temperature and the presence of other solutes, *i.e.*, upon the dielectric constant of the aqueous phase."

Dilatometric and other technique led to the conclusion that the more rapid the freezing of a gel, the greater is the damage it suffers and the less its subsequent affinity for water. Rapid freezing apparently removes part of the bound water, the system increasing in volume.

In isoelectric gels of concentration 43·7—52·1 per cent. gelatin, the bound water is about 0·56 gr. per 1 gr. gelatin, but it is less in weaker gels. During the freezing of the interstitial water of the latter, it is suggested that there is a mechanical tearing apart of a portion of the more loosely bound water from the gelatin aggregate, thus explaining the relatively large increase in volume when the gels are thawed.

Experiments with acidified gels led to the conclusion that the availability of water is a maximum at the isoelectric point, *i.e.*, bound water is a minimum. Directly opposite was the conclusion of Callow [1] based on the velocity of ice crystallisation in gels, but, as Moran points out, there was lack of equilibrium in Callow's experiments, whose velocities were probably related to variations in gel structure, *i.e.*, related to the state of aggregation of the gelatin-water complexes.

The problem of bound water in colloidal systems generally, but especially as related to biology, is attracting wide attention.[2] Gortner, who has been concerned with the subject for several years, has recently reviewed the position.[3] He mentions that the first suggestion of a bound water $\rightleftharpoons$ free water equilibrium (in relation to

[1] *Proc. Roy. Soc.*, **A 108**, 307 (1925).
[2] Hardy, *Proc. Roy. Soc.*, **A 112**, 47 (1926); *Kolloid-Z.*, **46**, 268 (1928).
[3] *Trans. Faraday Soc.*, **26**, 678 (1930). *Vide* also his "Outlines of Biochemistry' (New York, 1929), p. 227 *seq.*

certain pathological conditions) is due to Balcar, Sansum and Woodyatt.[1]

Newton and Gortner [2] independently proposed a bound water : free water theory, and devised a method based on the depression of freezing point to determine bound water. The method was applied by Newton [3] in his study of the winter hardiness of wheat whereby he concluded that winter hardy wheats showed a relatively large percentage of bound water. Non-hardy wheats were those unable to bind water when exposed to similar conditions of cold.

Summarising certain experimental data, Gortner concludes that water present on a colloid interface and not characteristic of water in bulk as commonly understood may be : (1) in the form of orientated water molecules at the interface, or (2) selectively adsorbed and orientated layers of H and OH ions forming an envelope round the micelle. To choose between these two notions is difficult, but it may be that adsorption of monohydrol alone constitutes the bound-water layer, the bulk water consisting of a mixture of associated water molecules. Further, there is not to be considered a sharp differentiation between bound and free water in colloidal systems ; rather must we " postulate an insensible gradation between molecules of water having the normal activity of pure water and molecules of water where this activity has been so reduced that such molecules have become to all intents and purposes a part of the solid upon which they are adsorbed."

Reverting to our discussion of the freezing of gels, we may note the extension of Moran's work by Hardy,[4] who made a microscopic study of the freezing of gels. He found that the progress of internal freezing is, as a rule, intermittent, except in very dilute gels. Freezing was followed by the microscope at — 7°, — 11° and — 12·7° C. and three types of freezing noted : (1) circles, corresponding to Moran's spheres, (2) rays, (3) disseminated.

Types 1 and 2 show optically homogeneous zones of doubly refracting solid solution separated by singly refracting membranes of gel 0·5 μ thick, produced intermittently. Type 3 shows separation of crystals of pure ice, each about 20 μ diameter and probably produced in continuous manner. For gels of 2—40 per cent. concentration, over the temperature range — 6° to — 13·5° C. freezing is of types 1 and 2, whilst with gels of less than 2 per cent. gelatin, more concentrated gels aged at least seven days, or gels of 15—40 per cent. gelatin and frozen at — 19° C., type 3 occurs. There is no spontaneous freezing at — 2·6° to — 3° C.

[1] *Arch. Internal Med.*, **24,** 116 (1919).
[2] *Botan. Gaz.*, **74,** 442 (1922).
[3] *J. Agr. Sci.*, **12,** 1 (1922) ; **14,** 178 (1924) ; Univ. Alberta Coll. Agr., *Res. Bull.*, **1,** 1—53 (1923). See also Thoenes, *Biochem. Z.*, **157,** 174 (1925) ; Bailey and Bayfield, *Cereal Chem.*, **7,** 108 (1930) ; Whitcomb and Sharp, *ibid.*, **3,** 301 (1926).
[4] *Proc. Roy. Soc.*, **A 112,** 47 (1926) ; *Kolloid-Z.*, **46,** 268 (1928).

Thawing leads to one of two results : (*a*) solid solution separates into ice and water at — 6° C. leaving a sponge of concentrated gel, and (*b*) at 0° C. there is sudden reabsorption of water by the gel surrounding the sponge.

Hardy concludes that the spongy structure resulting from thawing is not due to ice crystals as commonly believed. Moreover, Moran's suggestion that the ice phase consists of a mixture of ice crystals and particles of dehydrated gels finds no support from the optical behaviour of the gel nor its behaviour on thawing.

FREEZING OF HENS' EGGS

The thawing of frozen colloid systems does not usually restore the pre-frozen state, separation often taking place. With high rate of freezing recovery is sometimes attained on thawing, *e.g.*, chlorophyll solution frozen in liquid air. Moran [1] has made extensive investigations into this problem, using the complex colloid systems presented by hens' eggs.

He points out that in those systems where recovery is not attained on thawing, there exists a certain limited range of temperature (critical range) within which the desolution process can occur. " The peculiar feature of any temperature within the critical range, θ_1—θ_2, is that, if the system be kept at that temperature for a critical length (T) of time, changes take place which are not reversed on thawing."

T is probably a function of the form of the cooling curve, or, $T_\theta = f\left(\frac{d\theta}{dt}, \frac{d^2\theta}{dt^2}, \ldots\ldots\right)$, the function on the right hand side referring to the whole range $\theta_1 - \theta_2$.

Egg yolk in particular (egg white to a lesser degree) serves for experimental verification of the fact that the final state is a function of the entire temperature cycle, *i.e.*, the rate of thawing is all important, as well as the rate of freezing. Moran investigated this, studying the redistribution and change of state of the main protein of egg yolk and the volume changes occurring throughout the temperature cycle.

Egg yolk consists of rounded masses, ranging in diameter from 25 μ in the outer portion to 150 μ in the centre. It is a fine ground substance containing fat globules of various sizes. Each mass is semi-fluid and possesses a very delicate structure. The freezing point is — 0·65° C., whilst that of egg white is — 0·45° C.

Egg white is made up of two quite distinct portions, one thin and liquid and the other thick and gelatinous. The thick portion constitutes from 54 to 86 per cent. of the total egg white.[2]

[1] *Proc. Roy. Soc.*, **B 98,** 436 (1925) ; *Proc. 4th Intern. Cong. Refrig.* (*London*), **1,** 122 (1924).

[2] St. John and Green, *J. Rheology*, **1,** 484 (1930).

The changes occurring when egg white is frozen are less striking than when the yolk is frozen. At any temperature of freezing, the liquid portion increases and the viscous portion decreases, the latter probably losing water which is not completely reabsorbed on thawing.[1] The main factor controlling ice separation during freezing is the temperature attained during freezing, whilst the rates of freezing and thawing play very minor *rôles*. For precipitation or coagulation to take place prolonged freezing is essential, *e.g.*, four months at — 3° C.

When egg yolk is frozen in the temperature range — 0·65° C. to — 6° C., thawing yields normal fluidity. At temperatures below — 6° C. maintained " for a sensible time," the frozen yolk cannot be thawed to a liquid again, but only to a stiff paste. The critical range for such irreversible change commences at — 6° C., *i.e.*, $\theta_1 = -6°$ C. Again, there is a time factor. Although storage at — 6° C. for several months gives eggs which thaw reversibly, 20 hours at — 11° C. yield the pasty yolk on thawing. It is to be noticed as a remarkable fact that the change in the yolk occurs *when the yolk is frozen solid*, noting, however, that supercooling may readily be attained, *e.g.*, eggs at — 11° C. for 1 week may be thawed to yield normal yolks.

On freezing yolk in liquid air and then thawing at room temperature, the pasty form ensued, whereas rapid thawing in mercury at 30° C. produced normal yolk. Hence " the change of state under discussion may occur either during the fall or during the rise of temperature, and takes place in neither if the rate of change of temperature is high enough."

Since the irreversible change only occurs in the frozen material, it is deduced that it occurs in the concentrated solution of salts present in the yolk ; indeed, a critical concentration is indicated. Moran's experiments demonstrated that the irreversible change occurs with a solution of lecitho-vitellin in the concentrated salt present in frozen yolk below — 6° C., precipitation ensuing on thawing. The presence of 10 per cent. sucrose in egg yolk prevents the irreversible change and yolk of normal fluidity is gained even after freezing at — 11° C.

This " protection " of yolk is important, practically in the preparation of stable mayonnaise, and later experiments by Urbain and Miller [2] have shown that dextrose and levulose are far superior to sucrose in preventing lecithin coagulation after freezing at —15° C. for 72 hours. It was proved that there is no permanent combination of the sugars with any material in eggs during freezing. The explanation of such specific sugar protection remains for investigation, and the use of glycerol and mannitol should also be

[1] *Vide* Parsons, *Proc. 4th Intern. Cong. Refrig.* (*London*), **1,** 194 (1924).
[2] *Ind. Eng. Chem.*, **22,** 355 (1930).

considered. Certain experiments by Shrivastava, Gupta, Prasad and Bhatnagar [1] show that colloidal solutions of Cd, Sb, Se and As_2S_3 adsorb sugars and that the adsorbed sugar is no longer optically active. The present writer suggests that another variant of the problem would be the investigation of the possible effect of sugars and glycerol in inhibiting the coagulation of egg white and yolk on systematic shaking, *i.e.*, in inhibiting the Ramsden phenomenon (see p. 390).

Moran now followed the irreversible changes in egg yolk by the dilatometric examination of the volume alterations. Although there was no visible change in yolk supercooled to — 11° C., there was an irreversible change as demonstrated by a permanent decrease in volume. Such supercooling is rare and unstable.

The fertility of the egg of the common fowl is influenced by temperature. Moran found that the optimum temperature for maintaining fertility is from 8° to 10° C., whilst at about — 6° to — 7° C. the embryo dies at once. For the practical application of this work to the commercial storage of eggs reference should be made to the report of Moran and Piqué.[2]

FREEZING AND THAWING OF MEAT

The scientific problems involved in this very important commercial handling of meat are deep but full of interest and extensive modern work has been done and is yet in progress on various aspects, chemical and biological. Our discussion is necessarily confined to the colloid-physical aspects and must pass over the details of large-scale operations.

The fundamental problem which is presented concerns the marked structural changes in the tissues arising from freezing and thawing,[3] again the question of reversibility. On a practical scale it is a question of minimising " drip " of meat-juice when frozen meat is thawed for consumption. For example Pecori [4] has commented on the fact that rapid thawing at too high a temperature yields an unattractive meat, viscous and less digestible than normal. The exudation of liquid is enhanced, and about 0·6 per cent. of the albumin is lost. Up to the time of consumption the loss of liquid may amount to 10 per cent. Other investigators report similarly.[5]

[1] *J. Phys. Chem.*, **29,** 166 (1925); **28,** 730 (1924); Sen, *Z. anorg. allgem. Chem.*, **174,** 61,(1928) and *J. Indian Chem. Soc.*, **4,** 117, 131 (1927).

[2] Food Investign. Board, *Special Rept.*, No. **26,** 1—80 (London, 1926). *Vide* also Griffiths *et al.*, Dept. Agr. Union S. Africa, *Science Bull.*, **41,** 1—16 (1925).

[3] The student first approaching this subject is recommended to read: Moulton, " Meat Through the Microscope " (Chicago, 1929), pp. 21—32; Birdseye, *Ind. Eng. Chem.*, **21,** 416 (1929), both containing photomicrographs. Also Taylor, *Food Inds.*, **2,** 146 (1930).

[4] *Ann. igiene.*, **36,** 39 (1926).

[5] Fearon and Foster, *Biochem. J.*, **16,** 564 (1922); Clifford, *ibid.*, **19,** 998 (1925): Hoagland *et al.*, U.S. Dept. Agr., *Bull.*, **433,** 1—100 (1917); Barnicoat, *N. Zealand J. Sci. Tech.*, **11,** 409 (1930).

The edible portion of beef contains water, fat, proteins and salts, the analyses varying with the particular cut and the fatness of the sample. The approximate ranges (in percentage) for water, protein, fat and ash are 38—72, 9—21, 7—62, and 0·36—1 respectively.[1]

Since much of what follows concerns ordinary muscle a brief description of its physical structure is necessary. Muscle is built up of fibres, 1—2 inches long and about 0·002 inch in diameter. Sheathing the fibres is a membrane, the sarcolemma, which encloses the serum or aqueous system containing proteins, amino-acids and salts. Elastic sheaths of connective tissue unite the fibres into bundles.

RIGOR MORTIS

The well recognised condition of rigor mortis which is usually complete in beef in 24 hours, is the hardening and shortening of the muscles, resulting in a rigid carcase. Moran and Smith [2] have described the changes occurring in normal, intact muscle as: "(1) Hardening and stiffening, (2) shortening, (3) loss of elasticity, (4) loss of transparency, (5) loss of power to contract when electrically stimulated, (6) formation of lactic acid, and (7) production of heat [3] (heat of rigor)." After passing a maximum value, rigor decreases gradually, and in a few days the joints loosen and the muscles become flaccid. This is the phenomenon of the resolution of rigor.

Apparently these changes proceed parallel with increase in acidity, and this has led to the belief that rigor arises from coagulation of muscle substance by lactic acid.[4] However, Hoet and Marks [5] have demonstrated that a rapid and almost immediate rigor mortis occurs in rabbits succumbing to hypoglucemic convulsions or prolonged thyroid feeding. In this instance rigor is not due to acid formation in muscle but is due to the absence of glycogen and a decreased lactacidogen content of the muscle. There is neither shortening of the muscle nor opacity.

Moran and Smith [6] mention the early observations of Kühne (1864), who found that expressed muscle-substance is normally alkaline, but on standing at room temperature it becomes acid and forms a gel. However, the gel may also form without acid, and is then transparent; if acid is later produced, the gel becomes opaque and contracts with marked syneresis. The authors draw attention

[1] See Chatfield, "Proximate Composition of Beef," U.S. Dept. Agr., *Dept. Circ.*, **389**, 1—18 (1926).
[2] Food Investign. Board *Special Rept.*, **36**, 2 *seq.* (London, 1929).
[3] Smith, *Proc. Roy. Soc.*, **B 107**, 214 (1930).
[4] Bottazzi, *Atti. accad. Lincei.*, **1**, 68 (1925); de Caro, *ibid.*, **6**, 607 (1927).
[5] *Proc. Roy. Soc.*, **B 100**, 72 (1926).
[6] See Smith, *ibid.*, **B 105**, 579 (1930) for a detailed study on the coagulation of muscle plasma; also Report of the Food Investign. Board for Year 1929 (London, 1930), p. 13; *ibid.*, for 1927 (London, 1928), p. 16.

to the close correspondence of these two stages to the happening in the two types of rigor mortis and draw the conclusion " that rigor mortis normally consists of two processes occurring simultaneously : (*a*) gelation of the muscle-plasma, causing hardening and loss of response to stimulation ; (*b*) the production of acid, which causes contraction of the muscle, increase in opacity, and, later, the coagulation and breakdown of the protein gel, with the liberation of an acid serum."

FREEZING OF LIVING MUSCLE

An important communication by Moran [1] deals with the effect of cold and of simple desiccation on surviving muscle, and he confirms the hypothesis that the effect of cold on tissues is due to desiccation. Experiments were conducted with the sartorius and gastrocnemius muscles of the frog, the freezing point being — 0·42° C.

The freezing of sartorius muscle at — 4° C. or below produced rapid and pronounced shortening with accompanying exudation of acid fluid. Freezing at — 0·9° C. and — 1·5° C. produced no visible shortening, but freezing at — 2° C. for 48 hours led to 34 per cent. shortening. The shortening was more pronounced the lower the temperature of freezing, being 70 per cent. at — 10° C. and 80 per cent. after a few minutes immersion in liquid air.

Frozen muscles on thawing are found to have lost the osmotic properties of living muscle, and measurements of their changes in weight in 0·2 per cent. NaCl solution at 15° C. showed that : (*a*) freezing at — 1·5° C. for 48 hours does not impair the osmotic properties, (*b*) freezing at — 2° C. and lower irreversibly injures them. There is reason to fix about — 2° C. as a critical temperature, " and if it be defined as the temperature which in the frozen state permanently and practically at once destroys both the osmotic properties and the irritability of the sartorius, this critical temperature is independent of the time during which the frozen muscle is stored at it."

At temperatures intermediate between the freezing point and the critical point, injury to the muscle demands a time factor, this being less the closer the approach to the critical temperature.

Very rapid freezing (in liquid air) of sartorius muscle followed by rapid thawing (in warm mercury) caused the muscle to contract to a small acid non-irritable mass.

Experiments were now made on the effect of drying sartorius muscle in regard to irritability to an electric current. A loss in weight of 62 per cent. marked a critical point beyond which injury was irreversible. Calculated to dry weight, the critical value of water removal is 78 per cent., which corresponds to the freezing of the muscle at about — 2° C., equilibrium being attained.

[1] *Proc. Roy. Soc.*, **B 105,** 177 (1929).



FIG. 60.—Fresh beef muscle. (Mag. × 72.)

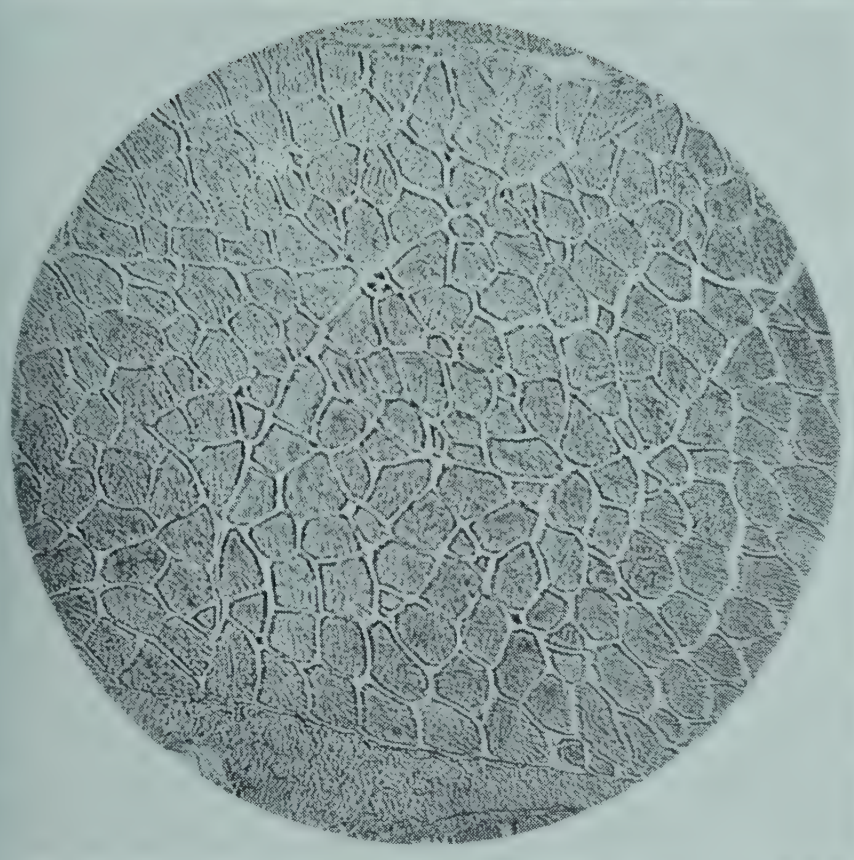


FIG. 61.—Transverse section of beef muscle frozen in liquid air. (Mag. × 72.)



FIG. 62.—Beef muscle frozen slowly. (Mag. × 33.)

To face p. 418.]

Moran summarises several arguments to correlate the effects of freezing and desiccation, and after noting several possible theories as to the mechanism of the injury effect, inclines to the view that it is due to an upset in ionic balance.

Smith [1] followed up this work by a study of the formation of lactic acid in muscles in the frozen state and after discussing Moran's results and possible explanations of the critical temperature he summed up as follows :—" A normal muscle with an adequate supply of oxygen is in a steady state, in which the rate of production of lactic acid and of its removal are so balanced that the percentage of acid present at any moment remains at a very low constant level. Freezing, that is, drying, upsets this balance by making the rate of production that of removal."

The disturbance in balance is caused by, or is concomitant with, injury to the mechanism. However, maintaining muscle at a temperature between the freezing point and — 1·6° C. for 24 hours does not destroy the mechanism of the removal of acid. Thus the accumulated acid is completely removed on thawing. Below —1·6° C. there is destruction of the mechanism of removal of acid, but the mechanism of the production of acid persists.

Moran later extended his investigations [2] to mammalian muscle post-mortem, seeking to supply data relating to the amounts of water existing as ice at different freezing temperatures in the case of beef, mutton and pork. The amounts of ice present in mammalian muscle when frozen to equilibrium at various temperatures in the range — 1·5° C. to — 20° C. were as follows :—

Equilibrium Temperature. °C.	Percentage of Total Water in the Muscle Present as Ice.
— 1·5	35·5
— 2·0	55·5
— 3·0	69·8
— 5·0	81·6
— 7·0	88·7
—10·0	94·0
—20·0	98·2

The results are interpreted to indicate that the amount of " bound " water in such muscle is small, not exceeding 6 per cent.

When muscle is frozen centres of crystallisation are formed, their manner and number being regulated by the rate and other factors of cooling. These centres are most active between the fibres,[3] and with normal cooling the result is that the fibres shrink, become distorted and are separated by masses of ice. Thawing, particularly if rapid, does not result in the melted ice becoming reabsorbed by the

[1] *Ibid.*, **B 105,** 198 (1929).
[2] *Ibid.*, **B 107,** 182 (1930).
[3] Moran, *J. Soc. Chem. Ind.*, **48,** 245T (1929).

muscle fibre. In the case of meat this water, as serum, causes "drip."

Slow thawing and storage at 1° C. after thawing, minimises this drip in beef, which is much more pronounced under similar conditions than with mutton.[1] Ultramicroscopical examination of the drips from beef and mutton was made by Vickery [2] who found that freezing and thawing brought about a reduction in the number of single colloid particles as compared with the unfrozen juices. As the rate of freezing decreased this reduction increased, but it was far more significant in the case of beef.

Very rapid freezing minimises drip. Again, veal shows the phenomenon to a much less extent than does old beef. Vickery also showed that under the same freezing conditions there is more separation of ice outside the muscle fibre in the case of beef than of mutton. This would be expected, since with sufficient rapid freezing that muscle richer in "bound" water (mutton) should exhibit the least ice separation outside the fibres.[3]

Since some water will always freeze outside the muscle fibres, the real problem is to study the conditions effecting the maximum reabsorption of such water on thawing. Such conditions are: (*a*) age of the animal, (*b*) temperature of the carcase and the time elapsing between slaughter and freezing, (*c*) rate of freezing, (*d*) temperature attained in freezing, (*e*) rate of thawing. Moran [4] believes that this problem must be considered on a molecular basis, investigating molecular breakdown in the muscle plasma and membranes (see Figs. 60, 61 and 62).

CHANGES IN MUSCLE-PIGMENTS

Hæmoglobin is the factor responsible for the colour of red muscle, either as muscle-hæmoglobin or as hæmoglobin present in those blood corpuscles residual in the capillaries. The former is present in an amount which varies with the type of muscle and the age and condition of the animal. Exposure of hæmoglobin to oxygen results in the slow formation of methæmoglobin, of dark brown colour.

Brooks [5] has investigated several factors involved in the production of methæmoglobin in pigmented muscles. The depth of penetration of oxygen into the tissue was studied and he found that slow formation of methæmoglobin took place in the oxygen region of tissue exposed to air, but maximum formation took place at some distance from the tissue/air interface.

Later, Brooks [6] extended the experiments, investigating the

[1] Cook *et al.*, *Australian J. Expil. Biol. Med. Sci.*, **3,** 15 (1926).
[2] *Ibid.*, **3,** 81 (1926).
[3] Report of the Food Investign. Board for 1925—26 (London, 1927), p. 23.
[4] *Ibid.*, p. 24.
[5] *Biochem. J.*, **23,** 1391 (1929).
[6] *Ibid.*, **24,** 1379 (1930). See also summary in Report of the Food Investign. Board for 1929 (London, 1930), p. 27.

effect of freezing in a concentrated solution of NaCl on the colour of red muscle. The red flexor muscles of the fore-leg of the ox were employed and exposure to oxygen invariably resulted in the formation of methæmoglobin in a superficial layer of tissue whether frozen or not. When air-frozen tissue was stored in air, the formation of methæmoglobin was very slow at low temperatures (56 days being required at — 20° C. for its spectroscopic detection), but freezing in brine or storage in aerated brine accelerated it considerably (5 days in brine at — 20° C.). "With thawed tissue which has been frozen in brine the increased rate of methæmoglobin formation at the higher temperature rapidly produced marked discoloration."

Freezing in brine with its accompanying penetration of NaCl into the tissue accelerates the oxidation of muscle—and blood—hæmoglobin to methæmoglobin. Just what is the mechanism of this oxidation is not fully worked out, but Brooks suggests that one factor is the pronounced increase in ionic strength which leads to an increased hydrogen ion activity, since other experiments using phosphate buffers only proved that a decrease in pH value increases the rate of oxidation.

FREEZING OF FISH

The preservation of fish by freezing is another important commercial undertaking [1] and one which is attracting considerable attention at the present time.

The chemical changes occurring when fish is frozen for periods ranging from 1 to 27·5 months were investigated by Clark and Almy,[2] using two species of salt water fish, *viz.*, bluefish (*Pomatomus saltatrix*) and weakfish (*Cynoscion regalis*). These were air frozen and held at about — 9·5° C. Keeping the fish "glazed" with ice maintained them in a palatable and wholesome condition for 16 and 13 months respectively. Glazing protects against loss of moisture, which normally would render the cold-stored fish uneatable after 4 months.

The water-soluble and coagulable nitrogen decreased in the case of the bluefish, whilst there was at first an increase and then a decrease in the case of weakfish, apart from those stored in the eviscerated condition and devoid of wrapping or glazing.

Later work by Clark and Field [3] took up the question of preserving fish frozen in chilled brine, of concentration 15—25 per cent. and at about — 11° C. The penetration of NaCl into the outer muscular layer $\frac{1}{8}$ inch deep varied according to the kind of fish used from 0·32 to 6·22 per cent. (on a dry basis). The keeping qualities

[1] Clark and Pennington, U.S. Dept. Agr., *Bull.*, **635**, 1—9 (1918).
[2] *Ind. Eng. Chem.*, **12**, 656 (1920).
[3] *Ibid.*, **13**, 927 (1921).

were excellent and instead of the fish losing weight, a slight gain was observed.[1]

A detailed discussion of the freezing of fish, including large-scale methods, has been given by Stiles [2] who points out that the question of freezing-injury rests on a similar basis to that involved in meat. In normal fish the flesh is made up of long muscle-fibres, bound into groups and separated by connective tissue. The sheath or sarcolemma encloses the protoplasmic material, rich in water. When freezing is conducted rapidly enough, tissue damage on thawing does not occur. Photomicrographs by Sherrill [3] show that quick frozen haddock possesses normal cells, whereas slow frozen haddock has the cells ruptured by ice crystals, resulting in waste when the fillet is thawed.

Stiles summarises important investigations made by Reuter [4] who froze fish at different rates, and observed the histological changes. Rapid freezing in liquid CO_2 did not change the appearance of the muscle of haddock and there was no separation out of ice. With less rapid freezing some water separated from the muscle protein and other colloids of the plasma whereby "a solid cylinder of ice comes to occupy the axis of each muscle fibre, while, owing to the expansion of water on freezing, the protein material is pressed against the sarcolemma." The latter, however, is not ruptured and moderately slow thawing thus permits the protein to re-absorb the water produced by the melting ice cylinders. With slow freezing damage to the muscle structure is evident, cavities appearing in the sarcolemma of the outer layers of fish, whilst in the interior of the fish the sarcolemma is ruptured and plasma escapes from the muscle fibres into the spaces between them.

In the case of air-frozen fish large ice crystals frequently become visible, water having separated from the muscle fibres into the connective tissue, only to be lost on thawing. In this connection the slower the rate of thawing the better, so that water re-absorption may be as complete as conditions will allow. With very rapid freezing, whereby the water freezes inside the muscle fibres themselves, the rate of thawing is not so important.

A comprehensive survey of the whole problem, with practical considerations, has recently been published by Zarotschenzeff.[5]

FREEZING FRUITS AND VEGETABLES

Comparatively little work has been done under controlled laboratory conditions regarding the changes taking place when

[1] *Ibid.*, **14**, 203 (1922).
[2] Food Investign. Board, *Special Rept.*, No **7**, 111—151 (London, 1922).
[3] *Food Inds.*, **1**, 33 (1928).
[4] See summary of Reuter's work by Nuttal and Gardiner, *J. Hyg.*, **17**, 56—62 (1918).
[5] "Between Two Oceans: Rapid Chilling and Freezing Systems for Fish and Meat" (London, 1930). Pp. 157.

fruits and vegetables are frozen. The subject deserves attention, if only from the standpoint of injury caused by frost, in the same way as fundamental problems are being attacked for animal systems.

The freezing point of apples and pears varies between — 1·1° C. and — 2·9° C., and the lowest temperature to which the fruit may be exposed without danger from alteration varies from — 1·4° C. to — 3·3° C. in the case of pears and from — 1·6° C. to — 2·58° C. in the case of apples.[1]

A chemical analysis of frozen oranges and lemons after very severe frost in 1913 was made by Young [2] who found the principal change to be an excessive loss of moisture. A slight but definite decrease in sugar and acid was also noted. Young advanced the view that the protoplasm of the cells in the fruit is killed by freezing and that the cell wall changes from a living, semi-permeable membrane to a dead, porous or sponge-like mass. The frozen fruit is characterised by connection being established between the rind and the pulp, so that water lost by evaporation from the former is replaced by the latter. The volume change of the fruit is slight and therefore, its specific gravity decreases.

Similar observations on lemons were made by Bailey and Wilson [3] who gathered frozen fruit and slowly thawed them out at 7°—10° C. Injured lemons showed visible shrivelling or desiccation. It was observed that the fruit which was left on the tree after freezing was poorer in juice and much poorer in acid than the fruit picked immediately after freezing and slowly thawed. Again, the fruit left on the tree decreased rapidly in specific gravity and were characterised by the formation of thick, puffy skins, shrivelled cells and hollow centres. The authors considered their results to confirm the common opinion that the injury to lemons following severe frost is not due to freezing but to rapid thawing. Slowly thawed lemons compared well with normal fruit in weight and appearance. Other investigators confirmed these results.[4]

Recent experiments by Carrick [5] have dealt with the freezing of grapes. The freezing point of Emporer grapes ranged from — 4·6° C. to — 1·8° C. The osmotic pressure of the cell juice in unfrozen grape pulp was 10·74 atmos. greater than in pulp frozen for 3 hours at — 8° C. The increase of respiration of Emporer grapes was pronounced when they were exposed for 1—2 hours at — 7·5° C., but 4 hours freezing did not greatly influence the normal CO_2 evolution.

Vegetables are also capable of being injured by freezing and data

[1] Aggradi, *Ann. chim. applicata.*, **20,** 188 (1930); Fawcett, *Rev. Ind. Agr. Tucuman*, **19,** 123 (1928).
[2] *Ind. Eng. Chem.*, **7,** 1038 (1915).
[3] *Ibid.*, **8,** 902 (1916).
[4] Webber *et al.*, Calif. Agr. Expt. Sta., *Bull.*, **304,** 245 (1919).
[5] Cornell Univ. Agr. Expt. Sta., *Mem.*, **131,** 3 (1930).

have been published relating to beets,[1] celery,[2] potatoes,[3] onions, etc. The latter have been investigated by Wright who found the average freezing point of the globe type of onions to be approximately — 1° C., and onions should not be stored below this temperature. Supercooling without actual freezing can readily occur, but a sudden jar or shock will at once induce freezing. The temperature to which supercooling may advance before freezing sets in is very variable among individual onions. If frozen onions are very slowly thawed, freezing injury becomes more pronounced than if they are rapidly thawed. They are not, however, so easily injured as potatoes, which are injured in 30 seconds after actual freezing sets in.

Wright[4] produces a coloured photograph illustrating the freezing injury in a yellow globe onion. "The affected tissue appears to be water-soaked, discoloured, and more or less transparent with indefinite scattered opaque areas. Symptoms of freezing injury are frequently and easily confused with those of physiological breakdown caused by excessive heat and other undetermined factors. When cut, such affected specimens show certain transparent discoloured scales similar to those caused by freezing injury, but usually without the scattered opaque areas. Freezing usually includes an entire scale all or nearly all the way around the onion. Often one scale is found injured while the adjoining ones may be normal. Following light freezing, usually the outer scale only is affected; with more severe freezing the injury is found in the more inner scales, often skipping one or two scales as it penetrates towards the centre. The last portion to succumb is the heart or growing point, at the centre of the base or receptacle."

[1] Vondrak, *Z. Zuckerind. cechoslovak. Rep.*, **50,** 130 (1925); *Intern. Sugar J.*, **28,** 149 (1926).

[2] Gonzalez, *Proc. Am. Soc. Horticult. Sci.*, **23,** 339 (1926).

[3] Wright and Taylor, U.S. Dept. Agr., *Bull.*, **916,** 1—15 (1921); Wright, Am. Assoc. Ice Refrig., *Proc. Ann. Mtg.* (1924/25), **14,** 119—23 (1925).

[4] Wright, U.S. Dept. Agr., *Dept. Circ.*, **415,** 1—8 (1927).

CHAPTER XIII

THE TREATMENT OF WATER AND FACTORY EFFLUENTS

THE CLARIFICATION OF WATER

WATER intended for use in the home and in the factory, particularly the food factory, is required to be clear, colourless and free from odour and taste and must conform to a desired standard of bacteriological purity.[1] The last item is beyond the scope of the present volume, but the modern methods of arriving at the physical and chemical standards of purity introduce problems of considerable interest to the colloid chemist.

Apart from well water, which is usually already clear and colourless and at once suitable for domestic use, water supplies are drawn from rivers and lakes or reservoirs and contain microbiological contamination and occasionally matter in colloid dispersion.[2] The removal of such contaminations is accomplished by colloid chemical means.

Natural water usually has a pH value from 7 to 8, buffered with $CaHCO_3$ and CO_2,[3] although bacterial growth may lower this pH value.[4] The purification of water begins with storage,[5] whereby the coarser suspended matter settles out and bacterial contamination is reduced, and is followed by a coagulation treatment prior to filtration. "Filter alum," *i.e.*, commercial aluminium sulphate, is the common coagulating agent employed, since on hydrolysis it yields $Al(OH)_3$ as a flocculent precipitate which removes impurities by mechanical entrainment as well as by adsorption.[6] In addition to this major factor, there are also the effects of the $Al^{\cdots}$ and SO_4'' ions to be considered. Negatively charged colloid matter as in turbid water, will be precipitated by the $Al^{\cdots}$ ions, and positively charged colloid

[1] See Christman, *J. Am. Water Works Assocn.*, **21,** 1076 (1929); Jordan, *Ind. Eng. Chem.*, **21,** 152 (1929).

[2] Christman, *J. Am. Water Works Assocn.*, **21,** 1076 (1929).

[3] Powers, *Ecology*, **10,** 97 (1929); Saunders, *Brit. J. Exptl. Biol.*, **4,** 46 (1926); Nasini and Porlezza, *Ann. Chim. applicata.*, **16,** 156 (1926), and *Atti. accad. Lincei.*, **5,** 622 (1927).

[4] Cf. Enevoldsen, *Biochem. Z.*, **181,** 251 (1927).

[5] *Vide* Bull and Darby, *Trans. Am. Inst. Chem. Eng.*, **18,** 365 (1927); Schlæffer, *Rev. Hyg.*, **47,** 1167 (1925); Weston, *J. New England Water Works Assocn.*, **39,** 225 (1925); Buswell, "The Chemistry of Water and Sewage Treatment" (New York, 1928), p. 151 *seq.*; Kolkewitz, *Ber. deut. botan. Ges.*, **46,** 35 (1928).

[6] Rakusin, *J. Russ. Phys. Chem. Soc.*, **48,** 97, 711 (1916); and *Z. Immunität.*, **34,** 155 (1922). Photomicrographs illustrating efficient clarification are given by McGrath, *Paper*, **35** (17), 44 (1925); *Paper Trade J.*, **80** (9), 46 (1925); *Paper Mill*, **46** (6), 80 (1925).

matter by the SO_4'' ions. Indeed, the $Al(OH)_3$ sol itself is flocculated by SO_4'' ions. Chlorine to the extent of about 0·6 p.p.m. is stated [1] to assist alum flocculation in water by its action on organic matter.

Wolman and Hannan [2] demonstrated theoretically and experimentally that the reaction of $Al_2(SO_4)_3$ with the natural alkalinity of water depends essentially on the hydrogen-ion concentration prevailing, complete precipitation of $Al(OH)_3$ occurring at approx. pH7, the flocs redissolving on either side of this pH value. Grobet [3] and others [4] have shown that the $Al(OH)_3$ is never precipitated pure, but always contaminated with aluminate. Thus, the addition of NaOH to dilute alum solutions gives successively : $Al(OH)_3$, $Al(OH)_3$. $Al(ONa)_3$ and $Al(ONa)_3$. Using potassium alum in litre quantities of a 0·005 solution and adding varying quantities of NaOH, Miller [5] found that "from the lowest pH value at which a precipitate forms up to a pH value of about 5·5 the composition of the precipitate remains constant and may be represented approximately by the ratio 10 Al : $3(SO_4)$. At higher pH values the sulphate content of the solid phase decreases and becomes zero above a pH value of about 9·0." The precipitate contains sulphate when there is less alkali than corresponds to the reaction : $Al''' + 3OH' \rightarrow Al(OH)_3$.

OPTIMUM FLOC FORMATION

The outstanding contribution to the problem of the optimum conditions for floc formation of $Al(OH)_3$ is that of Theriault and Clark [6] who found that this occurs over a narrow zone of pH which for dilute solutions is centred at pH 5·5, the zone narrowing more and more as the water is the more dilute in total salt content and the less is the amount of alum added. The latter varies considerably in actual practice, *e.g.* 1·5 grains/gal. at the Stuttgart Water Works,[7] 4 grains/gal. for Ontario R. Thames,[8] 3 grains/gal. for Ohio R. at Toronto,[9] 10 grains/gal. for Maumee R. at Toledo,[10] 0·75 grains/gal. for Tjiliwong R. in Batavia.[11]

Theriault and Clark observed that $Al(OH)_3$ which is precipitated at higher acidities can be gradually redissolved by adding alkali, and

[1] Gammage, *Eng. News Record*, **89**, 391 (1922) ; Weston, *J. Am. Water Works Assocn.*, **11**, 446 (1924).
[2] *Chem. Met. Eng.*, **24**, 729 (1921) ; **25**, 502 (1921).
[3] *J. chim. phys.*, **19**, 331 (1922).
[4] *E.g.*, Williamson, *J. Phys. Chem.*, **27**, 284 (1923).
[5] *Am. Coll. Symp. Monog.*, **3**, 209 (1925).
[6] U.S. Dept. Pub. Health, *Report*, **38** [5], 181 (1923).
[7] Egger, *Z. angew. Chem.*, **39**, 962 (1926).
[8] De Laporte, *Can. Eng.*, **46**, 316 (1924).
[9] Rupp, Ohio Conf. Water Purifcn., 4th Ann. Rept. (1925), p. 65.
[10] Furman, *3rd Ann. Rept., Ohio Conf. Water Purifcn.* (**1923**), 33.
[11] Mom, *Mededeel. Dienst. Volksgezondheid. Nederland-Indie*, Pt. **1**, 27 (1925).

concurrently there is a tendency for the crystalline form of the hydroxide to appear, a phenomenon noted by several investigators.[1] The authors have published titration curves (reproduced in Fig. 63) obtained from data when certain concentrations of potassium alum were titrated at 30° C. with Na(OH) solutions, determining the pH value after each addition of NaOH. The curve with blackened dots is the titration curve of a solution 0·02 M. with respect to Al, and that with circles corresponds to a solution 0·0002 M.

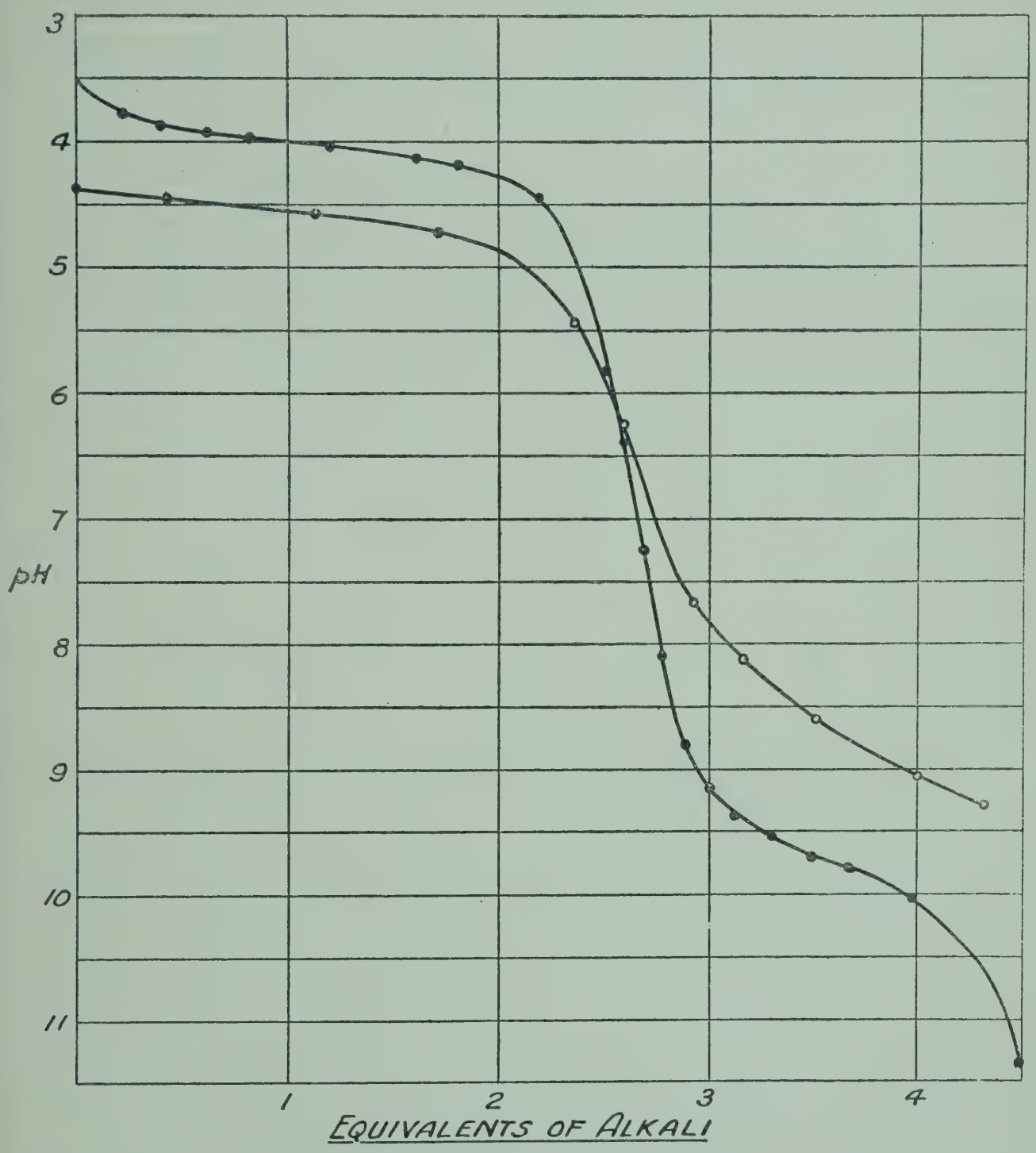


FIG. 63.—Titration curves showing the pH values of solutions of alum at successive stages of treatment with alkali.

The curves show an initial flat portion due to the precipitation of one or more constituents of the equilibrium during the progress of titration.

The optimum pH value for floc production in minimum time increased slightly as the alum concentration decreased, thus :—

[1] Fricke and Wever, *Z. anorg. allgem. Chem.*, **136**, 321 (1924); Böhm and Niclassen, *ibid.*, **132**, 1 (1923); Archibald and Habasian, *Trans. Roy. Soc. Canada*, **10**, 69 (1916); Slade and Polack, *Trans. Faraday Soc.*, **10**, 150 (1914).

Alum.	Optimum pH.
400 p.p.m.	4·95
300 ,,	5·10
200 ,,	5·25
100 ,,	5·40
<100 ,,	about 5·5

Graphs are also given showing the relation between the time for the first appearance of floc in solutions buffered at various pH values

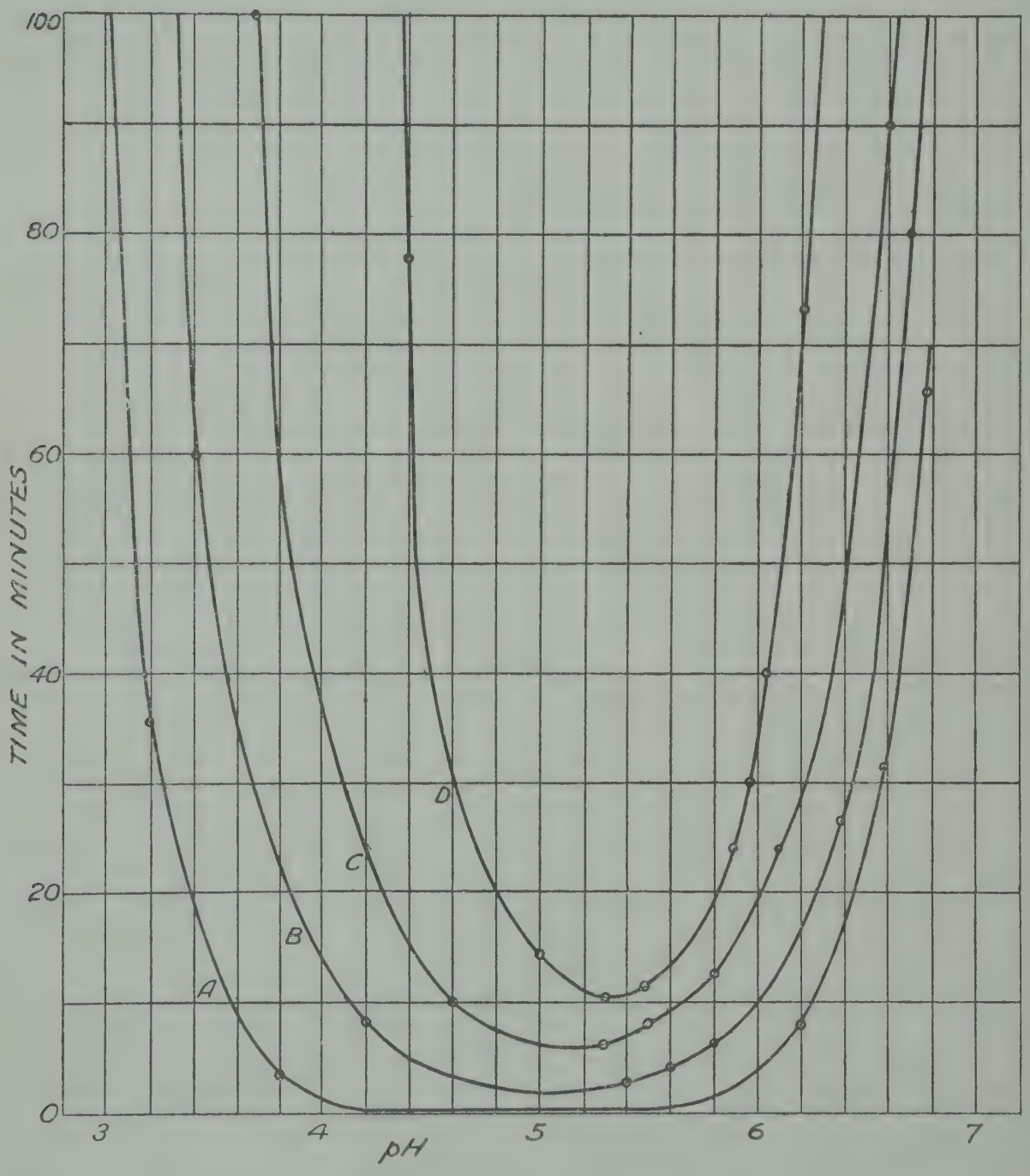


FIG. 64.—The relation between time required for the first appearance of floc in solutions buffered at various pH values when the total salt concentration is constant and the alum concentration is varied.

A = 400 p.p.m. of alum ; B = 300 p.p.m. of alum ;
C = 200 p.p.m. of alum ; D = 100 p.p.m. of alum.

when the total salt concentration is constant and the alum concentration varies (Fig. 64) and it is seen that as the alum concentration decreases, the width of the curves decreases. This means that operations with less than 50 p.p.m. alum require very

careful control of the final pH value in order to secure a floc in a reasonable time.

Varying the concentration of the buffer solutions used, Theriault and Clark found that on plotting time as ordinates and pH values as abscissæ, with constant (100 p.p.m.) alum content but varying total salt content (M/20 to M/200), there was a diminution in the width of the optimum zone as the concentration of the buffer (total salt) decreased. Accordingly, a water of low alkalinity or low total salt content demands more careful adjustment of alum dosage than do waters higher in alkalinity or total salt.

THE $Al(OH)_3$ FLOC

The solubility of $Al(OH)_3$ in cold water is given by Jander and Ruperti [1] as 0·6 mg. per litre. The solubility is a function of the hydrogen-ion concentration of the water, since $Al(OH)_3$ is amphoteric.[2] Various workers have studied this problem.[3] Thus, Greenfield and Buswell [4] find that $Al(OH)_3$ begins to precipitate at pH4 (Britton [5] reports pH 4·1), complete precipitation occurring at pH 6·5—7·5, with complete redissolving at pH 10—11. Hatfield [6] observed that the *minimum* solubility pH zone for $Al(OH)_3$ in carbonate and bicarbonate solutions extends approximately from pH 5·7—7·3, so that there would result the so-called "residual alum" or "soluble aluminium" if treated water falls near or outside these limits. The most favourable pH value or pH corresponding to minimum solubility is given as pH 5·5—6·0 by Mom,[7] whilst other workers identify this point as the isoelectric point of $Al(OH)_3$. Thus von Euler and Nilsson [8] give as the isoelectric point the value pH 6·6, and Hatfield [9] the value pH 6·1—6·3. The data of Heyrovsky,[10] used by Theriault and Clark, lead to the value pH 5·9, and these authors' own value is pH 5·6. These figures are not considered to be exact, and Theriault and Clark conclude that the pH value for the optimum coagulation with filter alum is between pH 5—6, whilst in buffered solutions, and with less than 100 p.p.m. of alum, they fix on the value about pH 5·5. Bachmann,[11] describing the influence of pH on the alum clarification of the municipal water

[1] *Z. anorg. allgem. Chem.*, **153**, 253 (1926).
[2] Britton, *J. Chem. Soc.*, **127**, 2120 (1925); Slade and Polack, *loc. cit.*
[3] Archibald and Habasian, *Trans. Roy. Soc. Canada*, **11**, 1 (1917); Massink, *Chem. Weekblad.*, **19**, 66 (1922); Bakhuyzen, *ibid.*, **19**, 41 (1922); Wagner and Enslow, *J. Am. Water Works Assocn.*, **9**, 373 (1922); Hatfield, *Ind. Eng. Chem.*, **14**, 1038 (1922); Banerji, *Indian J. Med. Research*, **11**, 695 (1924); Moser, *Helvetica Chim. Acta.*, **10**, 310 (1927); Carpenter, W. Va. Coll. Eng., *Tech. Bull.*, **1**, 55 (1927).
[4] *J. Am. Chem. Soc.*, **44**, 1435 (1922).
[5] *J. Chem. Soc.*, **127**, 2120 (1925).
[6] *Ind. Eng. Chem.*, **16**, 233 (1924).
[7] *Mededeel Burgerlijken Geneeskund. Nederland-Indie.*, Pt. **1**, 27 (1925).
[8] *Ber.*, **57**, 217 (1924).
[9] *J. Am. Water Works Assocn.*, **11**, 554 (1924).
[10] *J. Chem. Soc.*, **117**, 1013 (1920).
[11] *Rev. facultad. cienc. quim.*, **3**, 107 (1925).

supply of Buenos Aires obtained from the Rio de la Plata, states that alum is added to a pH value of 5·56—6·96.

Buswell [1] points out that such variations as the above show that $Al(OH)_3$ does not possess an isoelectric point in the same sense as the proteins but that there is a wide transition region between those pH values on the one side of which acidic ionisation predominates and on the other side basic ionisation. The one property common to the flocs in the intermediate zone is minimum solubility and the chemical characteristics of the $Al(OH)_3$ flocs form a factor in defining this zone.

An important paper by Miller [2] has confirmed and extended the work of Theriault and Clark. It is shown that in water purification by means of alum, there are three operating factors involved in successful clarification : (1) a minimum quantity of the $Al^{\cdots}$ ion is necessary, (2) there must be present an anion with pronounced coagulating power, (3) the hydrogen-ion concentration must be controlled. The concentration and valency of the anions present influence not only the chemical composition of the precipitate but also its physical and colloidal properties, *i.e.* its degree of dispersion in the flocculent condition.

The so-called alum flocs given by an aluminium salt and an alkali on interaction are not simply $Al(OH)_3$, except at high pH values, " but a more complex substance containing varying proportions of those anions present in solution." The composition of the floc thus varies with the pH value, and the width of the zone of the hydrogen-ion concentration in which flocculation occurs depends on the anion. Thus, in the case of alum, complete precipitation occurs over the range pH 5·3—8·7, whilst in the case of $AlCl_3$ such range is pH 7·8—8·6. Again, with alum, the supernatant liquor is clear, the SO_4'' ion having good coagulating power, whilst with $AlCl_3$ the supernatant liquor is cloudy, the Cl' ion being much less efficient in coagulating the positively charged $Al(OH)_3$ sol.

Miller thus refers to flocculation ranges, as more accurately describing the phenomena than the terms insolubility ranges or isoelectric ranges. He found, in agreement with Theriault and Clark, that the alum flocs were most dense, more opaque, less gelatinous and voluminous, and settled most rapidly, in the more acid portions of the flocculation range. His work serves to clear up the discrepancies in the results of pH values for optimum flocculation observed by previous investigators, since it is evident that flocculation is in part controlled by negative ions present in the raw water or subsequently added to it.

The effect of salts on the rate of coagulation and the optimum precipitation of alum floc has been investigated by Peterson and

[1] " The Chemistry of Water and Sewage Treatment," p. 162.
[2] *Am. Coll. Symp. Monog.*, **3**, 208 (1925).

Bartow,[1] who also employed electrolytes not found in natural waters but which served a theoretical interest. Using filter alum and NaOH, the point of optimum coagulation remained near pH 7·25—7·35, increase in alum dosage widening the pH zone, chiefly on the acid side, but the higher the alum dose the shorter was the time of coagulation.

The following table is based on the authors' tables and shows the effect of several salts on the minimum coagulation time of alum floc obtained from aluminium sulphate and NaOH.

TABLE 78. EFFECT OF SALTS ON MINIMUM COAGULATION TIME OF ALUM FLOCS.

Na_2SO_4.			Na. Oxalate.			K. Tartrate.			K_3PO_4.		
p.p.m.	pH.	mins.	p.p.m.	pH.	mins.	p.p.m.	pH.	mins.	p.p.m.	pH.	mins.
25	7·40	9	5	6·5—7·0	6·5	5	6·4	3	10	6·15	3
25	7·40	8	10	6·8	6·5	7·5	5·7—6·05	4	50	5·8	4·4
100	6·75	8	20	7·3—7·4	12	10	5·6	5·5	100	5·35	4
150	6·5—7·3	7	—	—	—	—	—	—	200	5·25—5·7	5·5
200	7·0	6									
500	5·5—6·9	4·5									

Regarding Na_2SO_4, with most waters of the middle west (100 p.p.m. or more of Na_2SO_4), in the absence of other factors, floc formation would occur over the pH zone 6·0—7·5, the optimum being about pH 6·75. Sodium oxalate in concentrations up to 5—10 p.p.m. decreases the coagulation time and extends the region of rapid coagulation up to pH 6·0. On increasing the oxalate, however, coagulation is slowed and is confined to the narrow pH zone 7·0—7·5. It is believed that a basic oxalate compound is formed with the alum flocs. A similar state of affairs arises with the tartrate ion. When phosphate is used, the optimum coagulation shifts to the acid end, from pH 6·25 (with 10 p.p.m.) to pH 5·3 (with 20 p.p.m.). Here again, the graphs do not represent simply the coagulation of $Al(OH)_3$, but more probably of a complex of this with phosphate.

The authors point out that since Theriault and Clark used phosphate buffers in their study of alum flocs, their results cannot be interpreted as showing the behaviour of pure $Al(OH)_3$. Emphasis is placed on the complexity of the alum floc in actual practice, this varying with different purification plants. Finally, although pH control is important, the chief controlling factor is the salt content of the raw water.

OTHER FACTORS IN FLOC FORMATION

Theriault and Clark have pointed out what is important in actual practice, that although in an homogeneous mixture of alum

[1] *Ind. Eng. Chem.*, **20**, 51 (1928).

and alkaline water, the reversible reactions proceed practically immediately to the equilibrium condition, the transition of $Al(OH)_3$ from the sol form to macroscopic flocs is a function of time, a fact also noted by Greenfield and Buswell [1] and studied on a large scale by Burdick.[2] The physical chemistry of floc formation in general is discussed by Werner,[3] and the rate of sedimentation by Tuorila.[4]

The minimum time required for the appearance of the $Al(OH)_3$ floc is, of course, identified with the point of optimum coagulation, pH 5·5. In a series of experiments where alum was treated with NaOH, Na_2CO_3 and $Ca(OH)_2$, Theriault and Clark noted that invariably the floc which appeared first had the best flocculent appearance and possessed "the qualities of rapid settling and abundance in the highest degree."

The time of flocculation is also a function of the size of the vessel in which the reacting solutions are mixed, it being invariably noted that flocs appeared in minimum time in the *large* vessels. Indeed, although a precipitate would form in a large vessel in a few minutes, hours would be necessary when employing small vessels. The deciding factor seems to be the effect of the volume—surface ratio upon circulation, since mechanical mixing hastens floc formation. Buswell [5] connects this with the low solubility of $Al(OH)_3$, the low concentration of the ions of which the precipitate is composed and the necessity of mechanical aid in bringing the particles into contact so that the micro-crystals can join together. A relatively slow wide-sweeping motion is particularly effective, but if the velocity is increased so that turbulence ensues, the efficiency diminishes. Raising the temperature would assist the flocculation, since the molecules would have greater kinetic energy and the viscosity hindrance would be lessened.

Hansen [6] also deals with this problem and mentions that at Highland Park, Mich., the mixing period is now 50 minutes. Again the tendency is for a longer period of settling following flocculation, 6 hours being frequently insisted upon, and even 18 hours being found to be advantageous. Occasionally, artificial turbidity is introduced into the water being treated so as to promote good flocculation.

Although the time factor is so evident, the total amount of floc produced is invariable, as is also the optimum pH, but the latter demands very rigid control, especially for very dilute solutions of alum.

[1] *J. Am. Chem. Soc.*, **44,** 1435 (1922).
[2] *Water Works Eng.*, **82,** 1629, 1633 (1929).
[3] *Kolloid-Z.*, **50,** 247 (1930).
[4] *Kolloidchem. Beihefte*, **24,** 1 (1927).
[5] "The Chemistry of Water and Sewage Treatment," p. 165.
[6] *Eng. News-Record*, **104,** 839 (1930).

TANNIC ACID AND ALUM FLOC

Of peculiar interest with regard to the clarification of coloured waters where tannic acid bodies are responsible for colour, are the experiments of Peterson and Bartow [1] who found that even small amounts of tannic acid greatly accelerate the coagulation of alum floc. Tannic acid and tannins are negatively charged colloids, hence their rapid flocculation with the positively charged alum floc. Optimum precipitation occurs at about pH 5·0—5·5, the zone being narrow and well defined. The floc is bulky and is probably complex in character. From this work it follows that the clarification of such waters as are coloured by tannin substances, must be effected in acid medium, the pH required depending on the tannin content. The following table by Peterson and Bartow illustrates the argument.

TABLE 79. EFFECT OF TANNIC ACID ON THE COAGULATION TIME OF ALUM FLOC.

Tannic Acid in Parts per Million.							
10		20		35		50	
pH.	Mins.	pH.	Mins.	pH.	Mins.	pH.	Mins.
5·5	23	5·1	25	5·0	20	4·75	22
5·7	10	5·3	14	5·3	5·5	4·85	10
5·8	5·5	5·95	3·5	5·5	2·5	5·15	2·5
5·95	4	6·1	3	5·7	1·5	5·45	1·5
6·05	3	6·2	2·5	5·8	1·5	5·43	1·5
6·2	2	6·4	2	6·1	1·5	5·65	2
6·3	2	6·6	2	6·2	1·5	5·85	2·25
6·4	2	6·7	2	6·6	2	6·0	6
6·6	2	6·8	2·8	6·76	9	6·25	28
6·7	2·5	6·9	8	6·85	20		
6·9	3	6·95	16				
7·1	4·5						
7·25	8						
7·45	20						

When using chlorinated copperas (p. 436) or ferric chloride, thus obtaining $Fe(OH)_3$ flocs instead of $Al(OH)_3$ flocs, Enslow [2] finds highly coloured swamp waters to be clarified best at pH 3·8—4·7, and experiments with tannin show maximum dispersion to occur at pH 8·2—9·2.

CLARIFICATION WITH SODIUM ALUMINATE

Several variants of the alum treatment of water have been proposed, such as double coagulation with alum [3] and the use of

[1] *Ind. Eng. Chem.*, **20**, 54 (1928).
[2] *J. Am. Water Works Assocn.*, **21**, 1602 (1929).
[3] Cf. Sheehan, *J. Am. Water Works Assocn.*, **12**, 442 (1924).

aluminium hydroxide sol,[1] 6 grains per gallon of the latter being useful in removing colour from water of low alkalinity. Recently, so-called sodium aluminate solution has been recommended by several workers and most favourably reported upon.[2] Barnitt and Haux [3] found that commercial sodium aluminate contained about 50 per cent. water-soluble Al_2O_3 and 35 per cent. Na_2O and Coxe [4] mentions its preparation by mixing a solution of 1 part Na_2CO_3 with a solution of 4 parts filter alum. Hedgepeth [5] proposes the use of sodium aluminate as an adjunct to that of alum.

The large-scale application of this reagent has been in use since March 1928 for the purification of the water supplied by the N.W. Branch of the Anacostia River for the Washington Suburban Sanitary District.[6] Maintaining the amount of the aluminate constant at 0·2 grains/gal., the dosages of alum and lime have been decreased and the necessity for pH adjustment with soda ash eliminated. Whereas without aluminate the average dose of alum was 1·25 grains/gal., this has fallen to 0·55 grains/gal. and the average lime dose has fallen from 0·88 to 0·3 grains/gal. Formerly, high turbidities necessitated the use of soda ash to the extent of 0·29 grains/gal.

The sodium aluminate is used in the form of a 2 per cent. solution and its advantages are claimed to be : (1) better floc formation, (2) rapid sedimentation of floc, (3) elimination of former troubles arising from presence of colloidal clay in the water. Previously the water left the plant at pH 8, so that the consumer had it at pH 7, lime having precipitated in the mains ; now the water leaves the plant at pH 7·4—7·6.

Similar results have also been reported by Ripple, Turre and Christman [7] who found it possible to reduce the alum dosage in the clarification of the Denver water supply (South Platte River) by means of sodium aluminate. Before the use of this reagent, a clear water was impossible with a dosage of alum under 6·8 p.p.m. but since employing aluminate (2·1 p.p.m.) the amount of alum falls below 6 p.p.m. and has even been as little as 0·9 p.p.m. The formation of floc is continuous and, although the size of the flocs is small, a more gelatinous floc results. Both the alum and the sodium aluminate contribute flocs. There is no residual alum in the treated water, the pH of which remains practically the same as that of the raw water. The service of the water filters has increased from the average of 24·6 hours to 29·8 hours, and the water required in filter

[1] Johnston and Downey, *Can. Eng.*, **50,** 94C (1926).
[2] Powell, *Am. J. Pub. Health,* **17,** 804 (1927).
[3] *Can. Eng.*, **51,** 725 (1926).
[4] *Chem. Met. Eng.*, **29,** 279 (1922). *Vide* also Spenser, *Chem. Age* (*N.Y.*), **32,** 31 (1924).
[5] U.S. Pat., 1,757,526 (1930).
[6] Morse *et al.*, *Ind. Eng. Chem.*, **20,** 56 (1928).
[7] *Ibid.*, **20,** 748 (1928).

cleansing has been reduced from the average of 2·58 per cent. to the average of 1·88 per cent. The average rate of filter operation daily has risen from 4·02 million gallons to 4·60 million gallons, whilst the former need of lime has been eliminated.

CLARIFICATION WITH IRON COMPOUNDS

Comparable in clarification efficiency [1] with $Al(OH)_3$ is $Fe(OH)_3$ and various means of precipitating this in water have been tried.[2] Ferrous sulphate, alone or in conjunction with alum, has been used with waters high in turbidity and low in colour and Peterson and Bartow [3] give the following figures relative to the effect of $FeSO_4$ on the coagulation time of alum floc where the alkalinity is adjusted with NaOH, and the alum dose is 34 p.p.m.

TABLE 80. INFLUENCE OF $FeSO_4$ ON THE COAGULATION TIME OF ALUM FLOC.

$FeSO_4$ in Parts per Million.							
0		10		25		50	
pH.	Mins.	pH.	Mins.	pH.	Mins.	pH.	Mins.
6·8	23	5·8	36	5·0	23·5	4·5	35
7·0	15	5·95	26	5·2	15·5	4·6	14
7·1	14	6·15	17	5·3	14	4·65	12
7·2	13	6·2	14·5	5·4	12	4·75	10
7·25	12	6·4	11·5	5·7	8	5·0	6
7·7	13	6·6	6	6·0	5	5·3	4
8·0	15	7·0	4	6·2	4·5	5·4	3·5
8·2	17	7·2	4	6·4	3·5	5·6	3
8·5	19	7·8	6	6·5	3	6·2	2
8·75	21	8·2	11·5	7·15	3	6·6	2
		8·6	23	7·6	3	7·2	2
		9·0	33	8·0	5	7·9	3
				8·25	7·5	8·5	8
				8·5	11	8·6	30

The ferrous sulphate effects pronounced reduction in the interval required for floc formation and extends the pH zone from pH 5 to pH 7·5. The authors point out that although ferrous sulphate would appear to be an ideal coagulation aid in waters low in total salts, and particularly in coloured waters since the colours are adsorbed best in acid reaction, the colours being tannin bodies which yield deeply coloured iron compounds, this renders its use doubtful.

Hopkins [4] has carried out experiments on the use of ferric

[1] *Vide* Kramer, *Z. Untersuch. Lebensm.*, **47**, 148 (1924).
[2] Cf. Wilson, *Eng. News-Record*, **104**, 1018 (1930).
[3] *Ind. Eng. Chem.*, **20**, 53 (1928).
[4] *Ibid.*, **21**, 58 (1929).

hydroxide floc for *turbidity* removal from Gunpowder River water. When 17—170 p.p.m. $FeSO_4$, $7H_2O$ are employed the floc is simply $Fe(HO)_3$, but at higher concentrations (428—1,710 p.p.m.) definite adsorption of sulphate occurs. The point of maximum hydroxide precipitation is pH 9·5 and the usual operating dosage of the ferrous sulphate (17—34 p.p.m.) secures this condition. Accordingly, good clarification of water occurs under economical plant operating values.

It should be noted that so long as the alkalinity, expressed in terms of NaOH, exceeds the stoichiometric amount required by the equation : $Fe^{\cdots} + 3OH' \rightarrow Fe(OH)_3$, the precipitate is sulphate-free and there is no formation of a basic sulphate as in the case of alum flocs. Again, the $Fe(OH)_3$ does not redissolve at high pH values, there being no soluble ferrate.

The river water contains iron (average 0·8 p.p.m.) and Hopkins furnishes the following table of pH values corresponding to the maximum removal of *iron* for different dosages of $FeSO_4$, $7H_2O$:—

$FeSO_4$, $7H_2O$ (p.p.m.)	8	17	34	51	68	86	171	428	1710
pH at max. precipitation	12·2	12·0	12·2	12·2	12·6	11·8	9·0	8·7	7·0

Operating at pH 9·4 provides efficient coagulation and, no matter what the original iron content of the water, the clarified supernatant liquid will not contain more than 1 p.p.m. of iron.

FLOCCULATION WITH CHLORINATED COPPERAS

When ferrous sulphate is hydrolysed as in water purification, the pH value for maximum flocculation is high, lime treatment being indicated to meet this requirement. Such high alkalinity is necessary in order to effect oxidation of ferrous hydroxide.[1] Oxygen is also required and these facts have led to the study of a ferric hydroxide floc obtained by the use of chlorinated ferrous sulphate (copperas).[2] Oxidation of ferrous sulphate with chlorine proceeds thus :—

$$6FeSO_4, 7H_2O + 3Cl_2 \rightarrow 2Fe_2(SO_4)_3, 7H_2O + 2FeCl_3.$$

With turbid water, chlorinated copperas gives a rapidly settling floc at any pH $> 3{\cdot}5$. It is capable of removing organic colouring matter from swamp water, but manganese is not adsorbed by the floc which is produced at a pH value $< 9{\cdot}4$, contrary to the usual iron and lime flocculation.

Hopkins and Whitmore [3] have shown that chlorinated copperas is an efficient coagulant for practical use, effecting excellent removal of turbidity with practically total precipitation of the iron and it can be employed to a degree impossible in the narrow alkaline zone

[1] Krause, *Z. anorg. allgem. Chem.*, **174**, 145 (1928). Cf. also Buswell, "The Chemistry of Water and Sewage Treatment," p. 176.

[2] Hedgepeth and Olsen, *J. Am. Water Works Assocn.*, **20**, 467 (1928); Enslow, *ibid.*, **21**, 1602 (1929).

[3] *Ind. Eng. Chem.*, **22**, 79 (1930).

(> pH 9·4) of the common iron and lime treatment. In the removal of colour from highly coloured water, analysis of the floc showed an organic complex therein, as would be expected on consideration of the adsorption of negatively charged coloured substances by the positively charged ferric hydroxide sol.

Regarding the composition of the floc, the authors give the following data :—

Chlorinated Copperas.	pH of Precipitation.	SO_4'' Adsorbed in Floc.
85 p.p.m.	3·8	+
	6·3	—
170 ,,	3·8	+
	6·2	—
425 ,,	3·6	+
	6·4	—
	7·3	—
	9·4	—
1,728 ,,	3·6	+
	6·6	+
	8·1	—
	9·4	—

The fact that no sulphate is adsorbed in the floc when the alkali used as a precipitant is less than stoichiometric is attributed to " the complete coagulation produced with the highly pre-oxidised ferric salt, at low concentration of electrolyte, in the presence of sufficient alkalinity to neutralise all the positive charges on the ion." But when large amounts of coagulant are employed (1,728 p.p.m.) below pH 7, there remain so many free positive charges that there are insufficient negative charges due to clays and other colloid impurities, and SO_4'' ions are required.

CLARIFICATION WITH FERRIC ALUMINA

Moberg and Partridge [1] have recently described the use of ferric alumina for water clarification, this being the hydrosol of aluminium hydroxide peptised by the addition of $FeCl_3$. The $Al(OH)_3$ is formed by neutralising an aluminium salt with alkali. The washed precipitate is mixed with $FeCl_3$ so that the ratio Al : Fe is 1 : 3.

The aluminium salt chosen is either the chloride or nitrate, *i.e.*, one wherein the anion possesses but slight coagulating power. Aluminium sulphate is impracticable since the coagulating power of the anion prevents dispersion to that degree which permits peptisation. The advantages of using $FeCl_3$ as the peptising agent

[1] *Ibid.*, **22**, 163 (1930).

are that the anion has weak coagulating power, the precipitate formed in subsequent reactions is heavier and therefore settles better than $Al(OH)_3$ alone, and the $FeCl_3$ itself being unstable in this connection, reaction proceeds with unusual ease.

Not all of the positive ions are coupled with negative radicals, so that the requirements of alkalinity for reaction become exceptionally low. Provided the molecular equivalents of Al to Fe are balanced, " the material will allow precipitation at virtually any pH common to water treatment, and in spite of any inhibitive colloids present."

Illustrating the advantages of ferric alumina, the authors mention a river water with appreciable coloured colloidal contamination, which required 2·5—3·5 grains/gallon of alum for flocculation (optimum pH 6·4), the alum dosage and pH control being exceedingly sharp. Using ferric alumina as coagulant, only 1—1·5 grains/gallon were needed, and coagulation occurred at pH 6·8—7·4, the pH of the river water itself. Again, water from Lake Michigan used at the city filtration plant at East Chicago, Ind., was most difficult to clarify owing to the daily fluctuations in quality. Alum (1·5—4·5 grains/gallon) with sodium aluminate (0·3—1 grain/gallon) proved not so reliable as ferric alumina (1—1·5 grains/gallon) for coagulation.

MISCELLANEOUS PURIFICATIONS

Barium aluminate has been proposed [1] as a useful coagulant in water treatment, since it would effect the simultaneous removal of sulphates, calcium, magnesium and colloidal contamination. The commercial product, a brown powder contaminated with $BaSO_4$, Al_2O_3, etc., on being made into a 10 per cent. solution yields a soluble portion of the approximate composition 11BaO, 8 Al_2O_3. With Ca or Mg salts precipitates are formed, 2 CaO or 2 MgO, with Al_2O_3, 6 H_2O, the former less soluble and the latter more soluble, as the temperature is raised. Pronounced anion effects are seen, the removal of calcium and magnesium being inversely proportional to the solubility of the barium salt produced during the reaction.

The well-known power of charcoal to adsorb substances from solution is frequently taken advantage of in water purification.[2] Granular charcoal with high adsorptive efficiently for chlorine is used to remove chlorine from water which has been treated with this gas for bacteriological purposes.[3] Organic chlorine compounds which possess undesirable odour or taste are also readily removed.[4]

[1] Stumper, *Chimie et industrie*, **22**, 1067 (1929).
[2] *Vide* a review by Bailleul, *Water Water Eng.*, **32**, 424 (1930).
[3] Koenig, *Gas Wasserfach.*, **72**, 1065, 1091 (1929).
[4] Jotten *et al.*, *Gesundh-Ing.*, **52**, 529, 546 (1929); **53**, 227 (1930).

Spalding [1] reports that as little as 0·5 grain/gallon of activated charcoal suffices greatly to reduce odours arising from cultures of *Oscillaria*, and if alum be used in conjunction (1·5 grains/gallon) the odours disappear. Again, the odour and taste due to phenol (1 p.p.m.) can be entirely removed by the use of the afore-mentioned quantities of charcoal and alum.

PURIFICATION BY ELECTROÖSMOSIS

Within recent years a new method of purifying water, even to the standard of distilled water, has been developed in Germany, and has received certain technical applications in relation to brewing and to ice manufacture, as well as to boiler water.[2] The method depends on electroösmosis, and in some instances on cataphoresis (of colloid matter) also.

In a two-phase system of solid and liquid, the application of an E.M.F. may lead to a relative displacement of the phases. Two cases demand consideration : (1) if the solid is in suspension and free to move, it may migrate to one of the electrodes ; (2) if the solid is fixed as a porous diaphragm the liquid may be forced through this. The first case is an instance of cataphoresis, and the second is electroösmosis or electrical endosmose.[3]

Several important papers discuss the specific application of electroösmosis to the purification of water.[4] The process developed by the Siemens-Elektro-Osmose G.m.b.H. really seems to be electrolysis [5] and not true electroösmosis, as the electrolytes are removed from the water owing to the migration of the anions and cations to the anionic and cationic compartments of the apparatus, whilst colloids are removed by cataphoretic movement.

The water undergoing treatment passes successively through a series of ten cells built up like a filter press. There are three compartments to each cell, the centre one being for the water undergoing purification, and the outer ones for the electrodes. Diaphragms permeable to ions and colloids separate the compartments, and by a syphon device the water in the centre compartment passes to the centre compartment of its neighbouring cell, and so on through the entire battery of cells, never mixing with the water in the electrode compartments, where the impurities accumulate and are washed away by a stream of water. As the purified water passes

[1] *J. Am. Water Works Assocn.*, **22**, 646 (1930).

[2] Cf. Bartow, *ibid.*, **22**, 115 (1930) ; Mayer and Schon, *Oesterr. Chem.-Ztg.*, **27**, 46 (1924).

[3] The phenomenon is described in detail by Freundlich, " Colloid and Capillary Chemistry " (London, 1926), p. 240 *seq.* ; Briggs, *Brit. Assocn. Coll. Repts.*, **2**, 26 (1921) ; Mayer in Liesegang's " Kolloidchemische Technologie " (Dresden, 1927), p. 94 *seq.* ; Chemnitius, *Chem.-Ztg.*, **53**, 361, 378 (1929).

[4] Illig, *Chem. Listy*, **23**, 408 (1929) ; *Z. angew. Chem.*, **39**, 1085 (1926). Behrman, *Ind. Eng. Chem.*, **19**, 1229 (1927) ; *J. Chem. Education*, **6**, 1611 (1929) ; *Chem. Age (N.Y.)*, **21**, 305 (1928) ; Patin, *Chimie et industrie*, **19**, 205 (928).

[5] Bartow and Jebens, *Ind. Eng. Chem.*, **22**, 1020 (1930).

through the apparatus its purity increases and, in consequence, its conductivity falls steadily, necessitating the application of a steadily increasing E.M.F. to meet this factor. The removal of colloids, if present, occurs in the later stages of purification under the higher E.M.F. imposed.

Describing the apparatus in detail, Illig[1] mentions that the anodes should be of magnetite and the cathodes of iron, zinc or tin. The diaphragms[2] are made from vulcanised fibre with the exception that at the anodes in the last chambers chrome gelatin is used. The cells are built from steel, rubber-coated, and the entire battery can deal with up to 250 litres of water per hour. Aten[3] investigated the process, applying 120 volts, with a current density of 0·2 amps. per cell. The water undergoing treatment flowed at the rate of 60 c.c. per minute, and after 24 hours he had obtained 72 litres of water of pH 4·5 and conductance 9×10^{-6}, and total solids 1—1·4 mgr. per 100 c.c. Similarly, Bartow and Jebens[4] purified water with an initial total residue of 725 p.p.m. to less than 15 p.p.m. and, as the residue was chemically inert, the water served for the common uses of distilled water.

In a 1,500 litre per day apparatus, using an E.M.F. of 110—220 volts (D.C.), von Bezold[5] obtained the following reductions in electrolyte content (gr./100 litres) : $CaCO_3$, 31·0—0·5 ; Cl, 1·0—*nil* ; SO_3 9·3 — 0·8 ; CaO 14·0 — 0·5 ; MgO 9·6 — 0·6. The power consumption was of the order 2 kw. hour per 100 litres of water containing 20 gr. electrolytes per 100 litres. The process is apparently uneconomical with water containing more than 1,000 p.p.m. of solid matter.

THE TREATMENT OF FACTORY EFFLUENTS

The problem of the disposal of effluents from food factories has considerable interest for the colloid chemist, since in addition to such biochemical methods[6] of treatment as aeration, septic tanks, Imhoff tanks, broad irrigation on the land, etc., chemical precipitation treatments are frequently employed and involve changes in the colloidal character of the effluent. The subject is an extensive one, and only certain broad outlines can conveniently be considered in

[1] *Siemens-Z.*, **8**, 349 (1928).
[2] Cf. Burkes, *Chem. App.*, **17**, 85 (1930) ; Remy, *Z. physik. Chem.*, **118**, 161 (1926).
[3] *Chem. Weekblad.*, **25**, 211 (1928).
[4] *Ind. Eng. Chem.*, **22**, 1020 (1930).
[5] *Brennstoff u. Wärmewirtschaft*, **8**, 242 (1926).
[6] See for example, Moor and Wayne, *Ind. Eng. Chem.*, **18**, 239 (1926) ; Bartow, *ibid.*, **18**, 1085 (1926) ; Mohlman and Beck, *ibid.*, **21**, 205 (1929) ; Hatfield, *ibid.*, **22**, 276 (1930) ; Underwood, *Ind. Chem.*, **3**, 260 (1927) ; Hennekine, *Bull. assoc. élèves inst. sup. fermentations Gand.*, **31**, 81 (1930) ; Walker, N.Y. State Sewage Works Assocn., Reprint 13 pp. (1929) ; Hommon, U.S. Pub. Health Service ; *Pub. Health Bull.*, **109**, (1921).

the present discussion, which will be limited in the main to : (*a*) dairy wastes, (*b*) cannery wastes, particularly from pea canning.

DAIRY WASTES

The disposal of milk waste effluents has special magnitude in the United States of America, and has received extensive study there. Since it is the protein matter which gives rise to the nuisance by its decomposition, the fundamental problem involved is that of the separation and disposal of protein, mainly casein. The pollution arising from dairy waste leads to a heavy black sludge of offensive odour. The casein decomposes, dissolved oxygen is completely exhausted and certain forms of algæ develop, *e.g.*, *Leptomitus lactis* and related species. Kimberley [1] has dealt with the dilution requirements of dairy waste for permissible stream loading, and states that the raw milk waste should be diluted 35—45 times, and occasionally as much as 100 times. Slaughter [2] advises a dilution not less than 50 times. Milk wastes may exert a toxic effect on fish in a stream, but more important is the fact that anaerobic conditions may arise in a stream consequent upon the absorption of the dissolved oxygen.

Dairy wastes, of course, differ considerably in composition, but an average analysis, according to Slaughter,[3] would be : 0·05—0·12 per cent. fat, 0·05—0·1 per cent. casein, 0·02 per cent. lactalbumin, 0·1—0·2 per cent. lactose, and 0·01—0·5 per cent. inorganic matter. Should the waste be untreated and permitted to acidify, casein will ultimately be precipitated at a pH between 4 and 5, and will rise to the surface, there undergoing bacterial decomposition, which yields offensive odours.

CHEMICAL PRECIPITATION OF DAIRY WASTE

Burroughs [4] investigated the use of $CaCl_2$, alum, alum + H_2SO_4, and H_2SO_4 as precipitants in the treatment of the wash water from a milk receiving station, containing approximately 0·25—0·5 per cent. of milk. The experiments aimed to avoid filtration so that sedimentation or precipitation became equal in importance to flocculation.

$CaCl_2$ gave no satisfactory floc over a temperature range of 12° C. to 50° C. Alum produced an excellent floc at pH 5·1, but its action is simply that of adjusting the pH, *i.e.*, alum acts in virtue of its acidic character. Using H_2SO_4 alone, the casein was precipitated over the pH range 5·1—4·6. For large-scale operations the author recommends H_2SO_4, the optimum precipitation of casein occurring over the pH range 4·9—4·5, and being independent of

[1] *Munic. Sanitation*, **1,** 89 (1930).
[2] Michigan State Coll., Eng. Expt. Sta., *Bull.*, **18,** (1928).
[3] *Loc. cit.*
[4] *J. Dairy Sci.*, **7,** 503 (1924).

summer and winter temperatures. Over-night sedimentation proves satisfactory, and, except in the hottest months, the settling tanks need cleaning but once a week. The method is cheap, requiring about 1 lb. of commercial H_2SO_4 of 50°—60° Baumé per 1,000 gallons of waste liquors.

Recently, Steel and Zeller [1] have studied the chemical precipitation of dairy wastes, using 0·5, 1, and 2 per cent. solutions of whole milk and skim milk. With H_2SO_4, for all three dilutions, optimum precipitation was observed over the pH range 5·4—4·8, so that the requirements of commercial H_2SO_4 per 1,000 gallons of 0·5, 1, and 2 per cent. whole milk waste would be 0·258, 0·386 and 0·755 lbs. respectively. The precipitation is very rapid and would permit of a continuous flow treatment providing 8 hours detention in the settling tank. On a large scale provision is necessary for easy removal of the considerable scum which forms. The clear effluent has a low biochemical oxygen demand.

Employing $FeSO_4$, it was found that lime is unnecessary. Steel and Zeller point out that the behaviour of ferrous sulphate in the case of milk wastes is not the same as when treating water where hydroxide flocs must form. On the contrary, the iron combines with casein yielding a heavy flocculent precipitate, which settles rapidly. Since acidity is the important factor in such precipitation, the addition of lime tends to inefficient working. Other workers, however, definitely recommend lime, as will be noticed later.

$FeCl_3$ proved a good precipitant, the optimum dosage in the case of 1 per cent. whole milk waste being 2·2 grains per gallon. Sodium aluminate being alkaline proved a failure. Filter alum was better than $FeSO_4$ and less was required. The addition of lime was unnecessary since the function of the alum is acidic. Table 81, adapted from the authors' data, shows the chemical dosages for the treatment of milk waste in water of low alkalinity.

TABLE 81. CHEMICAL DOSAGES FOR TREATMENT OF MILK WASTE.

Concentration of Waste.	H_2SO_4.		$FeSO_4$.		Alum.		$FeCl_3$.	
	Grains per Gal.	pH of Treated Waste.	Grains per Gal.	pH of Treated Waste.	Grains per Gal.	pH of Treated Waste.	Grains per Gal.	pH of Treated Waste.
0·5 per cent. .	1·4	5·0	5·0	6·0	1·5	5·5	—	—
1 ,, .	2·1	5·0	8·4	6·1	2·5	4·7	2·2	5·3
2 ,, .	4·1	4·9	11·7	5·7	3·3	4·7	—	—

[1] Texas Eng. Expt. Sta., *Bull.*, **38**, Series **4**, Vol. 1, No. 5 (1930)

Slaughter [1] has examined various methods of disposing of creamery wastes and finds that the septic tank is not well adapted for this, but that better results followed when enough lime is added daily to maintain the pH at 7·6, this reaction being the optimum for the bacterial reduction of the waste matter. Experiments were made using $FeSO_4$ and lime, adding solutions of these reagents in the order named, and excellent results obtained when 2 per cent. milk solution was treated with 0·5 grams per litre of $FeSO_4$ and lime then added to adjust the reaction to pH 7·8. A heavy gelatinous precipitate of hydroxide settles in about 10 minutes. "All suspended matter, including all the butterfat and most of the protein and bacteria, are carried down with the precipitate, leaving a slightly alkaline liquid containing about 900 p.p.m. of lactose, about 200 p.p.m. of dissolved protein, and about 600 p.p.m. of inorganic matter, consisting of dissolved ferrous hydroxide, excess calcium or sodium hydroxide and various salts." A typical analysis of the clear effluent is given as :—

	p.p.m.
Total solids	1,730
Inorganic solids	580
Organic solids	1,150
Fat	0
Suspended solids	0
Organic N	36

The effluent on storage gives rise to no odours, and, should secondary treatment in a sand filter be desired, it is ideal for the purpose.

Somewhat similar results have been reported by Eldridge [2] who advocates the addition of 4 lbs. $FeSO_4$ per 1,000 gallons of milk waste, with the further addition of lime to reach a pH of 9·4—10, optimal for the production of $Fe(OH)_2$ flocs. Sedimentation of 30 minutes is practicable. This worker also observed that alum used alone or with lime, NaOH or sodium aluminate gave poor results, gases forming at the optimum pH necessary for alum floc, and thus lifting part of the floc to the surface. Na_3PO_4 and $NaHSO_4$, without and with lime, produced a light flocculent precipitate, slow in settling and therefore devoid of practical interest.

PEA CANNERY WASTE

In the canning of green peas, it has been calculated that there is a loss of about 42 per cent. of the carbohydrate content of the peas. The main waste occurs : (1) as silage juice which seeps out of the heaps of pods after vining, (2) in the effluent from the blancher,

[1] *Loc. cit.*
[2] Michigan State Coll., Eng. Expt. Sta., *Bull.*, **24,** (1929) ; *Bull.*, **28** (1930).

where the peas are washed in hot water to remove their surface coating of gummy material.

Representative analyses of wastes from a pea cannery are given by Buswell, Greenfield and Shive [1] as :—

	Waste from Wash Troughs.	Blancher Liquors.		Wash Water from Blanched Peas.
		(*a*)	(*b*).	
Suspended solids . .	303	1,114	3,244	11
Turbidity	500	275	—	25
O_2 consumed from $KMnO_4$.	405	12,700	14,680	600
NH_3 nitrogen . . .	4·6	16	160	2·4
Albuminoid N . . .	60·0	300	160	84
Total organic N . .			2,240	
Total solids . . .			28,201	
Ash			3,550	

(The above quantities are in parts per million.)

Excellent clarification of pea cannery waste liquor was obtained by Warwick [2] using lime and $FeSO_4$. The initial pH value of the waste varied between 6·8 and 7·6. The lime dosage varied from 3·6—26·4 lbs. per 1,000 gallons, and the $FeSO_4$ dosage from 0·5—2·1 lbs. per 1,000 gallons. Table 82 collects some of the author's results.

In tests 1—5, where the lime is insufficient for complete reaction with the $FeSO_4$ to give satisfactory flocs, the treatment is not effective. Excellent results are given when the pH is not less than 10. The author's final recommendation is the application of 7·25 lbs. lime and 3·25 lbs. $FeSO_4$ per 1,000 gallons of wastes.

Daily cleaning of the settling tank is essential in order to avoid decomposition of the precipitate, since this would then rise as a scum and escape with the clear effluent. The sludge can be pumped on to drying beds and dried for fertiliser, a typical analysis of which is recorded as :—

	Per cent.
Moisture	41·33
Total N	0·50*
Total phosphoric acid	3·63*
Available phosphoric acid . . .	3·31*
Potash	0·00
Neutralising value (as $CaCO_3$) . .	32·8*

* Dry Basis.

The treated and clear effluent is disposed of by dilution in streams.

[1] *Ind. Eng. Chem.*, **18,** 1083 (1926).
[2] *Ibid.*, **19,** 577 (1927). See also N.Y. State Dept. Health, "The Treatment of Canning Wastes" (1930), p. 54.

TABLE 82. TREATMENT OF PEA CANNERY WASTE LIQUOR.

Test.	lbs. per 1,000 gal.		Treated Waste.		Reduction in Turbidity (per cent.).	Remarks.
	$FeSO_4$.	$Ca(OH)_2$.	pH.	Per cent. by vol. of Sludge.		
1	0·83	4·0	9·8	8	35·7	Tan floc ; poor coagulation and precipitation.
2	1·16	3·57	9·6	12·8	25	
3	1·84	4·14	8·8	9·2	55	Green and tan floc ; poor coagulation and settling.
4	1·96	4·3	8·9	13·6	48·4	Blue-green rapidly settling floc ; poor clarification.
5	1·37	13·8	9·2	12	68	
6	2·1	12·7	10	13·6	83·3	Brownish-green, rapidly settling floc ; very good clarification.
7	1·86	11·3	>10	16·8	91·7	
8	0·72	10·1	>10	12	93·8	Tan, rapidly settling floc, clear supernatant liquor.
9	0·56	11·4	>10	12	78·8	Tan floc, clarification only fair.
10	0·52	18·6	>10	12·8	90	Tan floc ; settling rapidly ; fair clarification.
11	0·89	21·3	>10	20	91·3	Tan floc ; settles rapidly ; good clarification.
12	1·87	25·4	>10	28	>92	Brownish-green floc, settling rapidly.
13	0·50	14·0	8·9	20	78	Dense, blue-green, rapid settling floc. Concentrated blancher waste.

MISCELLANEOUS WASTES

The use of lime and $FeSO_4$ is also advocated for treating the waste from tomato canning operations. The waste liquors are collected in treatment basins, dosed with lime and $FeSO_4$ (6 grains and 4 grains per gallon respectively) and permitted to settle in settling tanks overnight. The clear highly-coloured liquor is passed to sand filters and the sludge to drying beds.[1]

According to Kimberley,[2] an effective treatment is the addition of lime and sodium aluminate, using 2·8 grains and 17·5 grains per gallon respectively. He also found that 15—20 grains of filter alum, alone or with 5—8 grains of Na_2CO_3 per gallon gave rapid settling of raw, screened waste liquors from corn, lima beans and succotash. The clarified liquors, however, are peculiarly susceptible to bacterial decomposition, even in the presence of as much as 46 p.p.m. of chlorine.

Travers [3] recommends the treatment of cannery wastes in general with lime until alkaline, adding then a soluble calcium salt, followed by filter alum or $FeSO_4$ and a primary calcium phosphate. Lime is also used in the treatment of effluents from beet sugar factories,[4] optimum coagulation occurring at pH > 9. Application of chlorine[5] not only sterilises and deodorises such waste liquors, but by repressing fermentation, it assists lime coagulation since no protective colloids are formed.

Hedley [6] has described the chemical treatment of mill effluents from a Natal sugar mill. Milk of lime is employed, maintaining the alkalinity of the effluent at 0·6—1 part per litre, and then to each 500 parts of effluent is added 1 part of "alumina ferric." The precipitate settles readily and the sludge is filter-pressed. The clear liquor is yellow, very alkaline and free from odour.

[1] Webster, *Proc. 3rd Ann. Conf. Maryland Water Sewerage Assocn.*, **1929**, p. 33.
[2] Progress report on cannery waste treatment studies, Ohio State Dept. Health, Columbus, Ohio (1927).
[3] U.S. Pat., 1,711,105 (1929); 1,747,802 (1930).
[4] Cf. Lehmann, German Pat., 490,533 (1926).
[5] Jaskolski, *Listy Cukrovar*, **48**, 1, 9 (1929); Nolte, *Z. Ver. deut. Zuckerind.*, **79**, 163 (1929).
[6] *S. Afr. Sugar J.*, **14**, 317 (1930).

GLOSSARY OF TERMS

Absorption. The *general* penetration and internal accumulation of a liquid or gas by a system.

Adsorbent. The substance which adsorbs liquid or gas. Thus, charcoal adsorbs certain dyes from their solution, and gases from gas systems.

Adsorption. A change in concentration at an interface, *e.g.*, accumulation of gelatin at a benzene/aqueous solution of gelatin interface. Adsorption may be either positive or negative, representing increase or decrease in concentration respectively.

Adsorption Isotherm. Freundlich's expression connecting the extent of adsorption at constant temperature with the equilibrium concentration. Otherwise known as " the concentration function."

Amicron. Ultramicroscopic particles of diameter $< 1 \times 10^{-7}$ cm.

Amphoteric Electrolyte. An electrolyte, which by splitting off either hydrogen ions or hydroxyl ions as circumstances permit is capable of behaving as anion or cation. Amino-acids, *e.g.*, amino-benzoic acid, furnish notable examples.

Brownian Motion. The persistent zigzag motion of particles suspended in a liquid. The particles must not exceed about 4 μ diameter, whilst at about 0·1 μ diameter the motion is very pronounced, and is accompanied by considerable spatial displacement. (Named after the English botanist, Robert Brown.)

Capillary-Active. A solute capable of depressing the surface tension of a liquid (usually water). The term *surface-active* is synonymous.

Capillary Electric Phenomena. The electrical changes connected with changes in interfacial area or interfacial tension.

Cataphoresis. The motion of charged suspended particles in an electric field. The particles " wander " to that electrode, which has an electric sign opposite to that on the particles. Synonymous terms are : electro-cataphoresis and electrophoresis.

Coagulation. Synonymous with flocculation, signifying the removal of the factors which maintain colloidal systems stable. The most general coagulation phenomena refer to the removal of the electric charge on a colloid particle by the presence of suitable ions or a colloid of opposite charge.

Critical Potential. A term due to Powis for that potential which leads to flocculation of a colloid particle when the interface potential is lowered.

Denaturation of Proteins. *Apparently* irreversible chemical changes

of proteins *in solution* caused by heat, light, adsorption in foam, X-rays, alcohol, etc.

Dialysis. If a liquid system contains aggregates of various sizes, separation of these aggregates can be effected by virtue of their different rates of diffusion through a membrane (of suitable pore size) freely permeable to the liquid itself. The phenomenon of such separation is termed Dialysis.

Disperse Phase. The substance which is dispersed, *e.g.*, gold in an aqueous colloidal solution of gold, or oil in an oil-in-water emulsion.

Drop Number. The number of drops given by a standard volume of liquid when flowing from a pipette or tube into air or another (immiscible) liquid. The drop number serves as an approximate measure of the surface or the interfacial tension.

Electric Endosmose. The displacement of a *liquid* with respect to a *solid surface* under the influence of an applied E.M.F. The term is synonymous with Electrosmosis.

Electro-Dialysis. The separation of particles as in ordinary dialysis with the added advantage of accelerating the diffusion by imposing an E.M.F. on the system. In a general sense, it is a combination of dialysis and cataphoresis, whereby electrolytes are rapidly removed.

Emulsion. A system of two liquids, immiscible or nearly so, where one liquid is the disperse phase and the other is the dispersion medium or continuous phase. The two liquids are frequently spoken of as the internal phase and external phase respectively.

Emulsoid. A colloidal aqueous system characterised by an intimate relationship between the disperse phase and the continuous medium. Whereas suspensoids depend on electric charge for their stability, emulsoids depend on either electric charge or hydration or both factors together.

Flocculation. The coagulation of colloid particles due to the removal of the electric charge.

Gel. The term describes "transparent or translucent bodies containing a considerable fraction of liquid, but retaining their shape and exhibiting rigidity." (Hatschek.)

Gold Number (Zsigmondy). A *relative* measure of the protective action of emulsoids. The gold number is the minimum amount of emulsoid in milligrammes, which has to be added to 10 c.c. of a standard gold hydrosol in order to prevent the colour change (indicative of flocculation) when 1 c.c. of 10 per cent. NaCl solution is added.

Hydrophile. A marked affinity for water. The term lyophilic is synonymous. Speaking generally, the term has the same significance, in relation to colloid systems, as emulsoid.

Hydrophobe. Affinity for water is slight. The term lyophobe is synonymous and, in relation to colloid systems, has the same significance as suspensoid.

Hysteresis. Derived from the Greek word for *later*. The phenomenon of hysteresis, commonly encountered in investigations with

emulsoids, may be illustrated in the melting and setting of agar gels. Although a melted gel does not set on cooling until about 35° C., the set gel has to be raised to about 98° C. before it melts.

Imbibition. The taking up of a liquid by a gel.

Interface. The boundary separating two phases, *e.g.*, the liquid/liquid interface in emulsions and the liquid/gas interface in foams.

Interfacial Tensiometer. An instrument for measuring the interfacial tension between two immiscible liquids. Frequently, Du Noüy's apparatus is inferred, the interfacial tension being determined by the force required to pull a ring out of the interface.

Internal Phase. The substance which in a colloid system is the subdivided or disperse phase.

Iron Number. Analogous to gold number. Windisch and Bergmann define the iron number as that concentration of NaCl solution, which is just necessary in order to flocculate a ferric hydroxide hydrosol protected by a colloid, under standard conditions.

Isoelectric Point. The electric null point, *e.g.*, that hydrogen ion concentration at which a protein shows no cataphoretic motion in an electric field.

Jelly. A more "popular" and less rigid scientific term than gel, although practically synonymous with it.

Lyophile. See Hydrophile. The term is more general, as the nature of the liquid is not specified.

Lyophobe. See Hydrophobe. The term is more general, as the nature of the liquid involved is not specified.

Lyotropic Series. The arrangement of ions in series (Hofmeister series), in ascending or descending order of efficiency, relative to their effects on particular phenomena in colloid systems.

Membrane Equilibrium.—The distribution of ions in equilibrium when, for example, a membrane separates solutions containing common ions and non-common ions. Donnan's thermodynamic treatment of the various cases which arise has led to the frequent use of the synonymous term : Donnan equilibrium.

Micelle. Nägeli's term for the colloid particle. The term has been extended by Duclaux to apply to aggregates of ultramicrons and by McBain to such aggregates together with adsorbed ions.

Microns. Particles visible in the microscope. Particles of diameter $< 200\ \mu\mu$ are submicrons.

Monodisperse Systems. Systems containing particles of the same diameter.

Organosol. A colloidal system with an organic liquid as the dispersion medium.

Peptisation. The production of a colloidal solution by finer dispersion of a coarser system.

pH. Sørensen's logarithmic valuation of hydrogen-ion concentration, where $\mathrm{pH} = \log \frac{1}{[\mathrm{H}^{\cdot}]}$. Here $[\mathrm{H}^{\cdot}]$ signifies the concentration of hydrogen ion.

Polar Groups.—Groups such as —OH, —COOH, —CHO, —SH, —NCS which in an organic compound confer a degree of solubility in, or affinity for, water.

Polydisperse Systems. Containing particles of different sizes, the usual condition in colloidal solutions, especially lyophilic.

Protective Colloid. Usually refers to an emulsoid which aids the peptisation of hydrophobe sols, and assists in maintaining their stability against added electrolytes.

Ramdsden Phenomenon. The removal and denaturation of colloids (surface-active materials) in a froth because of adsorption at the air/liquid interface.

Rubin Number. A *relative* measure of the protective action of emulsoids. Wo. Ostwald's rubin number in that amount of the colloid in grams per 100 c.c. of solution which just prevents colour change in the dye, Congo-rubin, when 0·5 N. KCl is added under standard conditions.

Sensitisation. The phenomenon of an increased sensitivity to flocculation by electrolytes of hydrophobe sols, *e.g.*, when a non-electrolyte has been added.

Size Frequency Analysis. The determination of the number of particles in various groups in a colloid system, each group being based on a range of diameters.

Sol. The general name for a colloidal solution. The prefix indicates the particular liquid employed as the dispersion medium. Thus : hydrosol (water), glycersol (glycerine), alcosol (alcohol), organosol (organic liquid).

Sorption. McBain's term to include absorption and adsorption under the one phenomenon.

Stalagmometer. Traube's apparatus for determining the surface tension of liquids by the weight of drops.

Stoke's Law. The law of the *constant* velocity (V) of a small particle falling in a liquid. If :

r = the radius of the particle,
d = the specific gravity of the particle,
d' = ,, ,, ,, ,, liquid.
η = the viscosity of the liquid,
g = the gravity constant,

$$V = \frac{2r^2(d - d')g}{9\eta}$$

Submicrons. Particles visible in the ultramicroscope but not visible in the ordinary microscope.

Surface-Active. See Capillary-active.

Suspensoids. Colloids, which in colloidal solution are sensitive to the action of electrolytes. The suspensoids, *e.g.*, colloidal metals, are differentiated from emulsoids in this respect. The term lyophobe is preferable.

Syneresis. The phenomenon of the spontaneous exudation of fluid from a gel.

Tensiometer. An instrument for measuring the surface tension of liquids.

Thixotropy. The phenomenon shown by certain colloidal systems of changing their condition on being stirred or shaken. A concentrated hydrosol of ferric oxide is a notable example; at rest a condition of rigidity is noticeable, whilst stirring restores the sol condition.

Traube's Rule. Surface-active materials of an homologous series show surface-activity increasing in pronounced and regular manner as the series is ascended.

Tyndall Effect. The scattering of light at the surface of colloid particles dispersed in a liquid. A sol, clear in transmitted light, shows a turbid path when a beam of light is projected through it, the sol being viewed at right angles to the direction of this beam.

Ultracentrifuge. An apparatus devised by Svedberg enabling increased sedimentation of colloidal solutions owing to the imposition of enormously increased gravitational force.

Ultrafiltration. Filtration of colloidal solutions through special filters designed to hold back the colloid material. Ultrafilters may be prepared to retain colloid particles of different diameters.

Ultramicroscope. An instrument which recognises colloidal units by making visible the Tyndall light diffracted from the unit.

Xerogels. "Gels, formed by drying, and very poor in liquid, but nevertheless coherent." (Freundlich.)

BIBLIOGRAPHY

AGAR

Büchner, E. H., and Kleijn, D. The Flocculation of Agar Sols Through Salt Mixtures. *Proc. Acad. Sci. Amsterdam*, **30**, 740—2 (1927).

Calàbek, J. The Swelling of Biocolloids : Ultraviolet Rays and Swelling of Agar. *Protoplasma*, **3**, 17—42 (1927).

Carey, C. L. The Gross Structure of an Agar Gel. *Bull. Torrey Botan. Club*, **48**, 173—82 (1921).

Clarke, B. L. The Swelling of Agar Gels as a Function of Water Content before Swelling. *J. Am. Chem. Soc.*, **47**, 1954—8 (1925).

Cunningham, J. Preparation of a Purified Agar Powder with Increased Powers of Filtration. *Indian J. Med. Research*, **6**, 560—2 (1919).

Dokan, S. The Action of Electrolytes in the Swelling of Agar. *Kolloid-Z.*, **34**, 155—61 (1924) ; Swelling and Viscosity of Japanese Konyaku, *Ibid.*, **35**, 11—18 (1924).

Effront, J. The Absorbent Power of Agar. *Compt. rend.*, **180**, 29—33 (1925).

Fairbrother, F., and Mastin, H. The Swelling of Agar-Agar. *J. Chem. Soc.*, **123**, 1412—24 (1923).

Fellers, C. R. Some Bacteriological Studies on Agar-Agar. *Soil Science*, **2**, 255—90 (1916) ; Analysis, Purification and Some Chemical Properties of Agar-Agar. *Ind. Eng. Chem.*, **8**, 1128—33 (1916) ; The Nutritive Value of Agar-Agar and Its Use as a Jellying Medium. *Pure Products*, **13**, 177—85 (1917).

Free, E. E. Swelling of Gelatin and Agar Gels in Solutions of Sucrose and Dextrose. *Science*, **46**, 142—3 (1917).

Friedman, L. Structure of Agar Gels from Studies of Diffusion. *J. Am. Chem. Soc.*, **52**, 1311—14 (1930).

Harvey, E. H. Physico-Chemical Study of U.S.P. Agar. *Am. J. Pharm.* **97**, 447—56 (1925) ; The Electrodialysis of Agar. *Ibid.*, **97**, 66—70 (1925).

Hatschek, E., and Humphry, R. H. Certain Physical Differences between Sols and Gels of Agar. *Trans. Faraday Soc.*, **20**, 18-22 (1924-25).

Hatschek, E. Intensity of the Tyndall Light in Agar Gels as a Function of the Degree of Hydration. *Kolloid-Z.*, **48**, 246—8 (1929).

Healey, D. J. The Exudate from Nutrient Agar Slants : The So-called Water of Condensation. *J. Bact.*, **12**, 179—80 (1926).

Hoffman, W. F., and Gortner, R. A. The Electrodialysis of Agar. A Method for the Preparation of the Free Agar Acid. *J. Biol. Chem.*, **65**, 371—9 (1925).

Iwase, K. Coagulation of Agar Hydrosol. *J. Tokyo Chem. Soc.*,

41, 468—79 (1920); Electrical Conductivities of Salt Solutions Containing Agar. *Bull. Chem. Soc. Japan*, **2**, 61—5 (1927).

Jong, H. G. B. de. The Viscosity Change at the Commencement of Gelatinisation of Dilute Agar Sols. *Rec. trav. chim.*, **47**, 797—818 (1928); Capillary-Electric Charge and Hydration as Condition Variables in Hydrophile Gels: Reversible Volume Changes of Agar Gels. *Kolloidchem. Beihefte*, **29**, 454—83 (1929).

King, J. The Adulteration of Conserves with Special Reference to Pectin and Agar-Agar. *Analyst*, **50**, 371—83 (1925).

Krishnamurti, K. Scattering of Light in Colloidal Solutions and Gels (I.) Agar Sol and Gel. *Proc. Roy. Soc.*, **A 122**, 76—103 (1929); with **Donnan, F. G.** The Scattering of Light in Sols and Gels. *Am. Coll. Symp. Monog.*, **7**, 1—16 (1930).

Kruyt, H. R., and Jong, H. G. B. de. The Lyophilic Colloids: (I.) General Introduction: Agar Sol. *Kolloidchem. Beihefte*, **28**, 1—54 (1928).

Lüdtke, M. Studies on Agar. *Biochem. Z.*, **212**, 419—29 (1929).

MacDougall, D. T., and Spoehr, H. A. Effects of Acids and Salts on Bio-Colloids. *Science*, **46**, 269—72 (1917); The Effect of Organic Acids and Their Amino Compounds on the Hydration of Agar and on a Bio-Colloid. *Proc. Soc. Exptl. Biol. Med.*, **16**, 33—5 (1918); The Solution and Fixation Accompanying Swelling and Drying of Bio-Colloids and Plant Tissues. *Plant World*, **22**, 129—37 (1919); Swelling of Agar in Solutions of Amino Acids and Related Compounds. *Bot. Gazz.*, **70**, 268—78 (1920).

Mackinnon, H. D. Agar, A Colloid of Importance Takes its Place as a Food Adjunct. *Food Inds.*, **2**, 123—7 (1930).

Prát, S. Antagonistic Salt Action in Relation to the Swelling and Dyeing of Powdered Agar. *Am. J. Botany*, **14**, 167—86 (1927).

Robertson, G. R. The Agar Industry in California. *Ind. Eng. Chem.*, **22**, 1074—7 (1930).

Samec, M., and Ssajevič, V. The Composition of Agar. *Compt. rend.*, **173**, 1474—5 (1921); Physicochemical Analysis of Agar Gels. *Kolloidchem. Beihefte*, **16**, 285—300 (1922).

Tressler, D. K. Edible Algæ and the Agar-Agar Industry. "Marine Products of Commerce" (New York, 1923), Chap. 6.

Waele, H. de. Gelation of Agar. *Ann. physiol. physicochim. biol.*, **5**, 877—80 (1929).

Yanagawa, T. A Study of Agar-Agar. *Repts. Imp. Ind. Research Inst. Osaka Japan*, **10**, No. 6 (1929).

IRISH MOSS

Field, I. A. Source, Preparation and Properties of Algal Gelatins. *Chem. Age (N.Y.)*, **29**, 485—6 (1921).

Gloess, P. The Industrial Exploitation of Marine Plants. *Mon. sci.*, **6**, 97—108 (1916).

Gutbier, A., and Huber, J. Studies of Protective Colloids (XI.). Carragheen as Protective Colloid: (A) General Colloid-Chemical Investigation of the Extract of Irish Moss. *Kolloid-Z.*, **30**, 20—31 (1922).

Haas, P. Carrageen (Chondrus crispus) : The Occurrence of Ethereal Sulphates in the Plant. *Biochem. J.*, **15,** 469—76 (1921) ; The Nature and Composition of Irish Moss Mucilage. *Pharm. J.*, **106,** 485 (1921).

Haas, P., and Hill, T. G. Carrageen, Chondrus crispus. *Ann. Appl. Biol.*, **7,** 352—62 (1921).

Haas, P., and Russell-Wells, B. Irish Moss Mucilage and a Method for its Determination. *Analyst*, **52,** 265—9 (1927) ; The Hydrolysis of Carrageen Mucilage. *Biochem. J.*, **23,** 425—9 (1929).

Harwood, F. C. The Colloidal Electrolyte Extracted from Carrageen (Chondrus crispus). *J. Chem. Soc.*, **123,** 2254—8 (1923).

Jong, H. G. B. de, and Gwan, O. S. Lyophilic Colloids : (VI.) Electroviscous Effect with Two Hydrophilic Sols which do not Obey Poiseuille's Law ; Linseed and Carrageen. *Kolloidchem. Beihefte,* **29,** 436—53 (1929).

Justin-Mueller, E. Some Peculiar Reactions of Carrageen Moss Towards Certain Dyes Used Therapeutically. *J. pharm. chim.*, **30,** 154—61 (1924).

Rankin, C. The Use of Irish Moss as Copper Finings. *J. Inst. Brewing,* **35,** 287—91 (1929).

Russell-Wells, B. Carrageen (Chondrus crispus) : The Constitution of the Cell Wall. *Biochem. J.*, **16,** 578—86 (1922).

Sauvageau, C. Gelose and the Algæ which Produce It. *Bull. sci. pharmacol.*, **29,** 637—41 (1922).

GUMS

Amy, L. Chemistry of Gum Arabic. *Bull. soc. chim. biol.*, **10,** 1079—90 (1928).

Bungenberg, H. G., and Gwan, O. S. Studies on Complex Coacervation (2) Serum Albumin and Gum Arabic. *Biochem. Z.*, **221,** 182—205 (1930).

Clark, G. L., and Mann, W. A. A Quantitative Study of the Adsorption in Solution and at Interfaces of Sugars, Dextrin, Starch, Gum Arabic and Egg Albumin and the Mechanism of Their Action as Emulsifying Agents. *J. Biol. Chem.*, **52,** 157—82 (1922).

Dannerth, F. The Industrial Chemistry of Chicle and Chewing Gum. *Ind. Eng. Chem.*, **9,** 679—82 (1917).

Ditmar, R. The Manufacture of Chewing Gum as a Problem in Dispersion. *Gummi-Ztg.*, **40,** 156—8 (1925).

Ewing, C. O. Karaya Gum, a Substitute for Tragacanth. *J. Am. Pharm. Assocn.*, **7,** 787—90 (1918).

Gabriel, L. F. The Effect of Heat on Acacia. *J. Am. Pharm. Assocn.*, **19,** 828—30 (1930).

Garner, W. The Properties and Analysis of Gums. *Ind. Chemist,* **3,** 341—4 (1927).

Giral, M. J. The Mucilages. *Rev. gén. coll.*, **8,** 9—22 (1930).

Greenish, H. G. The Production of Gum Acacia. *Pharm. J.*, **105,** 265—6 (1920).

Gutbier, A., Huber J., and Krivoss, Z. Gum Tragacanth as Pro-

lective Colloid : General Colloid-Chemical Investigation of Tragacanth Mucilage. *Kolloid-Z.*, **18,** 141—52 (1916).

Hamy, A. Characterisation of the Gum of Acacia Verek. *Bull. sci. pharmacol.*, **35,** 421—2 (1928).

Hart, R. Carob-Seed Gum. *Ind. Eng. Chem., Anal. Edn.*, **2,** 329—31 (1930).

Krantz, J. C. The Buffer Capacities of Acacia and Tragacanth. *J. Am. Pharm. Assocn.*, **18,** 469—73 (1930).

Kruyt, H. R., and Tendeloo, H. J. C. Lyophilic Colloids : Gum Arabic Sol. *Kolloidchem. Beihefte,* **29,** 396—412 (1929) ; Charge, Hydration and Particle-Size in Sols of Starch, Gum Arabic, Gelatin and Casein. *Ibid.*, **29,** 413—31 (1929).

Locher, E., Kreidl, K., and Gutbier, A. General Colloid-Chemical Investigation of Locust-Kernel Gum. *Kolloid-Z.*, **33,** 37—9 (1923).

Razman, H. Physical and Chemical Nature of the Natural Gums. *Farben-Ztg.*, **20,** 639—40, 667—9 (1915).

Ridgway, J. A. Gum Arabic. Its Collection, Marketing and Chemical Characteristics. *Can. Chem. J.*, **4,** 330—4 (1920).

Schwartze, E. W., Cretcher, L. H., and Hann, R. M. Pharmacology of Arabic Acid and Acacia. *J. Pharmacol.*, **33,** 275—6 (1929).

Taft, R., and Malm, L. Solvents for Gum Arabic. *Trans. Kansas Acad. Sci.*, **32,** 49—50 (1929) ; Some Physico-Chemical Properties of Gum Arabic-Water Systems and Their Interpretation. *J. Phys. Chem.*, **35,** 874—92 (1931).

Thomas, A. W., and Murray, H. A. A Physico-Chemical Study of Gum Arabic. *J. Phys. Chem.*, **32,** 676—97 (1928).

Trotman, S. R. The Vegetable Gums. Valuation of the Commercial Products. *Chem. Trade J.*, **82,** 500—1, 525—7, 601—2 (1928).

Voigt, J. Investigations on Gum Arabic with the Aid of Silver and Gold Sols. *Kolloid-Z.*, **54,** 307—10 (1931).

Walter, H. Measurement of the Viscosity and Surface Tension of an Emulsoid (Gum Arabic). *Sitzb. Akad. Wiss. Wien.*, Abt. II. A. **129,** 709—20 (1920).

Went, S. The Viscosity of Gum Acacia Solutions Together with a Determination of the Viscosity Constant of Such Solutions According to the Formula of Arrhenius $\log \eta = \theta$ C. *Am. J. Physiol.*, **85,** 458—67 (1928).

Williams, A. L. Locust-Kernel Gum and Oil. *Analyst,* **53,** 411—5 (1928).

PROTEINS

Abramson, H. A. The Ionisation of Adsorbed Protein. *Proc. Soc. Exptl. Biol. Med.*, **26,** 689—90 (1929).

Abramson, H. A., and Grossman, E. B. A Method for the Rapid Dialysis of Large Volumes of Proteins. *J. Gen. Physiol.*, **14,** 487—91 (1931).

Amar, J. Bound water in Colloids. *Compt. rend.*, **186,** 1147—9 (1928).

Ando, K. Hofmeister Ionic Series in the Precipitation of the Hydrophilic Colloids. *Nagoya J. Med. Sci.*, **2,** 110—23 (1927).

Anson, M. L., and Mirsky, A. E. On Some General Properties of Proteins. *J. Gen. Physiol.*, **9**, 169—78 (1925).

Artom, C. The Precipitation of Various Concentrations of Proteins with Neutral Salts. *Arch. sci. biol. Italy*, **14**, 391—420 (1930).

Bancroft, W. D., and Barnett, C. E. Solid Protein Hydrochlorides. *Proc. Nat. Acad. Sci.*, **16**, 118—23 (1930); Adsorption of Ammonia by Proteins. *Ibid.*, **16**, 135—9 (1930); Phase Rule Studies on the Proteins: (I.) Determination of Solid Compounds with HCl or NH_3. *J. Phys. Chem.*, **34**, 449—98 (1930); (2) *Ibid.*, **34**, 753—98 (1930); **34**, 1217—53 (1930); (4) **34**, 1930—46 (1930). With **Belden, B. C.** Compound Formation with a Volatile Base or Acid. *Am. Coll. Symp. Monog.*, **7**, 151—60 (1930).

Benedicenti, A., and Bonino, G. B. Ionic Activity in Protein Systems. *Arch. sci. biol. Italy*, **14**, 293—326 (1930).

Bergmann, M. The Structure of Proteins. *Naturwissenschaften*, **17**, 314—6 (1929).

Bogue, R. H. Hydrogen Ion Concentration and the Properties of the Emulsoid Colloids. *J. Phys. Chem.*, **26**, 801—11 (1922).

Bottazzi, F. The Surface Tension of Colloids with Special Reference to Protein Colloids. Alexander's "Colloid Chemistry," Vol. 2, 121—64 (New York, 1928); Surface Tension of Protein Solutions. *Am. J. Physiol.*, **90**, 291—2 (1929).

Buchner, E. H. The Hofmeister Series and the Hydrogen Ion Concentration. *Rec. trav. chim.*, **49**, 1150—60 (1930).

Caro, L. de, and Laporta, M. Surface Tension of Protein Solutions. *Rend. accad. sci. Napoli.*, (4), **35**, 171 (1929); Changes in the Surface Tension of Protein Solutions as a Function of pH. *Arch. sci. biol. Italy*, **14**, 264—92 (1930).

Coca, A. F. The Dialysability of Proteins. *J. Immunol.*, **19**, 405—10 (1930).

Cohn, E. J., Gross, J., and Johnson, O. C. The Isoelectric Points of the Proteins in Certain Vegetable Juices. *J. Gen. Physiol.*, **2**, 145—60 (1919).

Cohn, E. J. Studies in the Physical Chemistry of the Proteins: The Solubility of the Proteins at Their Isoelectric Points. *Ibid.*, **4**, 697—722 (1922).

Csonka, F. A., Murphy, J. C., and Jones, D. B. Isoelectric Points of Various Proteins. *J. Am. Chem. Soc.*, **48**, 763—8 (1926).

Desai, S. V. Investigations on the Separation of the Digestion Products of the Proteins by the Method of Differential Dialysis. *Biochem. J.*, **24**, 1897—1904 (1931).

Dhéré, C. The Colloid-Physical Chemistry of Proteins. Remarks on the Work of Jacques Leob according to Personal Observations. *Ann. physiol. physicochem. biol.*, **2**, 95—102 (1926); Electrodialysis in Biochemistry. *Bull. soc. chim. biol.*, **8**, 144—59, 604—10 (1926); *Kolloid-Z.*, **41**, 243—58, 315—26 (1927). The last paper contains a bibliography of 90 references.

Donnan, F. G., and Krishnamurti, K. The Scattering of Light in Sols and Gels. *Am. Coll. Symp. Monog.*, **7**, 1—16 (1930).

Eckweiler, H., Noyes, H. M., and Falk, K. G. The Amphoteric Properties of Some Amino Acids and Peptides. *J. Gen. Physiol.*, **3**, 291—308 (1921).

Ettisch, G. The Conduct of Amphoteric Substances in Their Adsorption on Collodion Membranes. *Naturwissenschaften*, **18**, 447—8 (1930).

Fikentscher, H., and Mark, H. The Viscosity of Lyophilic Colloids. *Kolloid-Z.*, **49**, 135—48 (1929).

Fischer, M. H. On the Theory of Lyophilic Colloids and the Behaviour of Protoplasm. *Am. Coll. Symp. Monog.*, **1**, 244—64 (1923).

Fodor, A. The Colloid Chemistry of the Proteins. *Kolloid-Z.*, **30**, 313—36 (1922).

Gorter, E., and Grendel, F. The Spreading of Proteins. *Proc. Acad. Sci. Amsterdam*, **32**, 770—1 (1929).

Gortner, R. A., Hoffman, W. F., and Sinclair, W. B. Proteins and the Lyotropic Series. *Kolloid-Z.*, **44**, 98—108 (1928) and *Am. Coll. Symp. Monog.*, **5**, 179—98 (1928).

Greenberg, D. M. Some Aspects of the Electrochemistry of the Proteins. *Trans. Am. Electrochem. Soc.*, **54**, 107 (1928).

Harris, L. J. Zwitterions (I.) Proof of the Zwitterion Constitution of the Amino-Acid Molecule. *Biochem. J.*, **24**, 1080—5 (1930) ; with **Birch, T. W.** (2) Amino-Acids, Polypeptides, etc. and Proteins as Zwitterions, with Instances of Non-Zwitterion Ampholytes. *Ibid.*, **24**, 1086—97 (1930).

Hartl, K. and Starlinger, W. Physico-chemical Behaviour, as Dissolved Sodium Salts, of Electro-Dialytically Purified Protein Groups from Human Blood- and Exudate-Plasma. *Z. ges. exptl. Med.*, **60**, 289—314, 315—35 (1928).

Haugaard, G., and Johnson, A. H. Fractionation of Gliadin. *Compt. rend. trav. lab. Carlsberg*, **18**, (2), 1—132 (1930).

Heymann. E. Proteins and Electrolytes. Thermochemical Investigations. *Kolloid-Z.*, **50**, 97—105 (1930).

Hitchcock, D. I. The Ionisation of Protein Chlorides. *J. Gen. Physiol.*, **5**, 383—94 (1923).

Höber, R., and Schürmeyer, A. Ultramicroscopic Observations of Ion Antagonism in Protein Solutions. *Pflüger's Arch. ges. Physiol.* **214**, 516—23 (1926).

Hoffman, W. A., and Gortner, R. A. Physico-Chemical Studies of Proteins : The Prolamines—Their Chemical Composition in Relation to Acid and Alkali Binding. *Am. Coll. Symp. Monog.*, **2**, 209—368 (1925).

Ito, T., and Pauli, W. Electrolyte-Free Proteins : Free Charge and Behaviour to Neutral Salts of Purest Proteins. *Biochem. Z.*, **213**, 95—108 (1929).

Jermolenko, N. Concerning the Optical Properties of Protein Sols as Affected by Neutral Salts and pH. *Kolloid-Z.*, **54**, 66—73 (1931).

Johnston, J. H. St. Surface Tension of Protein Solutions. *Biochem. J.*, **21**, 1314—28 (1927).

Jong, H. G. B. de, and Dekker, W. A. L. Flocculation and Separation. The System Gum Arabic-Gelatin. *Biochem. Z.*, **213**, 318—36 (1929).

Klarmann, E. "Die Rolle der Zyklischen Amino-Saure-Anhydride in der neuren Strukturchemie der Proteine." (Berlin, 1929).

Klobusitzky, D. v. The Effect of Hydrogen Ion Concentration on the Salting Out of Serum Proteins. *Biochem. Z.*, **223**, 120—9 (1930).

Kolthoff, I. M. The Chemical State of Protein Solutions. *Chem. Weekblad.*, **22**, 489—94 (1925).

Kondo, K., Kinsuke, K., and Hayashi, T. Studies on Proteins. (A) On the Preparation of Rice-Glutelin. *Mem. Coll. Agr. Kyoto Imp. Univ.*, **2**, 37—53 (1926); (B) On the Point of Optimum Flocculation of Rice Glutelin. *Ibid.*, **2**, 55—69 (1926); (C) The Influence of Salt on the Point of Optimum Flocculation of Rice Glutelin. *Ibid.*, **2**, 71—81 (1926); (D) On the Isoelectric Point of Glutelin. *Ibid.*, **5**, 1—47 (1928).

Kondo, K., and Ito, T. Globulins of Polished Rice. *J. Chem. Soc. Japan*, **51**, 67—70 (1930).

Kroepelin, H. The Thermodynamics of Lyophilic Colloids. *Kolloid-Z.*, **53**, 86—8 (1930).

Kruyt, H. R. The Colloidal State of Protein Solutions. *Chem. Weekblad.*, **22**, 473—5 (1925); Unity in the Theory of Colloids. *Am. Coll. Symp. Monog.*, **5**, 7—18 (1928).

Kuhn, R. The Theory of the Isoelectric Point. *Z. physik. Chem.*, **114**, 44—8 (1924).

Linderstrøm-Lang, K. The Ionisation of Proteins. *Compt. rend. trav. lab. Carlsberg*, **15**, 1—29 (1924).

Lloyd, D. J. The Biological Functions of the Proteins. *Biol. Rev.*, **3**, 165—77 (1928).

Loiseleur, J. The State of Biochemical Substances, especially Proteins, in Anhydrous Solution. *Compt. rend.*, **191**, 1391—3 (1930).

Lüers, H., and Schuster, K. Colloid Chemistry of Yeast Proteins. *Kolloid-Z.*, **32**, 334—7 (1923).

Mardles, E. W. J. The Solubility of Proteins. *Trans. Faraday Soc.*, **26**, 750—60 (1930).

Mark. H. Physical Basis of the Naegeli Micellar Theory. *Naturwissenschaften*, **19**, (45/47), 892—900 (1928).

Michaelis, L. Isoelectric Point of Electroamphoteric Colloids. *Nernst's Festschrift* (Halle, 1912), 308—18.

Michaelis, L., and Nakashima, T. An Additional Method for the Determination of the Isoelectric Point of Proteins and Its Application to Serum Albumins of Animal Origin. *Biochem. Z.*, **143**, 484—91 (1923).

Modern, F. Protein and Acids. *Anales asoc. quim. Argentina*, **25**, 160—86 (1927).

Nord, F. F. Biological Significance of the Physical State of Lyophilic Colloids. *Trans. Faraday Soc.*, **26**, 760—7 (1930).

Noüy, L. P. du. The Surface Tension of Biological Solutions. *Protoplasma*, **6**, 494—516 (1929).

Ostwald, Wo., and Hertel, R. H. Colloid Chemical Reactions between Protein Sols and Polymerised Carbohydrates. *Kolloid-Z.*, **47,** 258—68 (1929).

Pauli, W. Proteins as Colloids. Alexander's "Colloid Chemistry," Vol. 2, 223—34 (New York, 1928); Electrolyte-Free Water Soluble Proteins: Hydration and Charge of Protein Ions. *Biochem. Z.*, **202,** 337—64 (1928); Some Advances in the Colloid Chemistry of the Proteins and Their Biological Significance. *Klin. Wochcshr.*, **8,** 673—9 (1929); Constitution and Electrochemical Behaviour of Proteins. *Kolloid-Z.*, **53,** 51—61 (1930); The Behaviour of Proteins Towards Colloids and Towards Electrolytes. *Trans. Faraday Soc.*, **26,** 723—37 (1930).

Pauli, W., and Valkó, E. "Elektrochemie der Kolloide." (Vienna, 1929).

Pauli, W., and Weiss, E. Reactions between Colloids: Dyes and Proteins. *Biochem. Z.*, **203,** 103—41 (1928).

Pfeiffer, H. The Isoelectric Point of Cells and Tissues. *Biol. Rev., Camb. Phil. Soc.*, **4,** 1—40 (1929); *Trans. Faraday Soc.*, **26,** 822—34 (1930); "Elektrizität und Eiweisse insbesondere des Zellplasmas" (Dresden, 1929).

Prideaux, E. B. R., and Howitt, F. O. The Electrophoresis of Protein Sols in the Presence of Gold Sols—Albumin, Gelatin and Casein. *Proc. Roy. Soc.*, **A 126,** 126—37 (1929).

Rawlins, L. M. C., and Schmidt, C. L. A. Combination between Basic Dyes and Proteins. *J. Biol. Chem.*, **82,** 709—16 (1929).

Reinders, W., and Bendien, W. M. Influence of Lyophilic Colloids on the Stability and Electric Charge of Lyophobe Sols. *Rec. trav. chim.*, **47,** 977—88 (1928).

Robertson, T. B. The Combination of Proteins with Acids and Bases with Some Observations on the Origins of Viscosity in Protein Solutions. Alexander's "Colloid Chemistry," Vol. 2, 255—302 (New York, 1928).

Roche, G. Physico-Chemical Properties of Natural Globulin. *Compt. rend.*, **189,** 378—80 (1929).

Rona, P., and Weber, H. H. Mechanism of the Specific Action of Ions on Proteins. Degree of Activity of Different Myogen Salts. *Biochem. Z.*, **203,** 429—51 (1928).

Sandstrom, W. M. Physicochemical Studies on Proteins. *J. Phys. Chem.*, **34,** 1071—1101 (1930).

Saunders, F. Studies in Proteins: Concerning the Uniformity of the Protein Fraction Extracted from Orange Seed Meal by Salt Solutions. *J. Am. Chem. Soc.*, **53,** 696—700 (1931).

Shibata, K. On a New Conception of the Constitution of Proteins. *Acta Phytochimica*, **2,** (4) 193—8 (1927); *Proc. Intern. Congr. Plant Sci.* (Ithaca, 1926), **2,** 1190—3 (1929).

Simms. H. S. The Nature of the Ionisable Groups in Proteins. *J. Gen. Physiol.*, **11,** 629—40 (1928).

Sørensen, S. P. L. Protein Studies. *Z. physiol. Chem.*, **103,** 1—14 (1918); Solubility of Proteins. *J. Am. Chem. Soc.*, **47,** 457—69 (1925); Composition and Characterisation of Genuine Proteins.

J. Chem. Soc., **129**, 2995—3009 (1926); The Constitution of Soluble Protein Substances as Reversible, Dissociable Component Systems. *Kolloid-Z.*, **53**, 102—24 (1930); **53**, 170—99 (1930); **53**, 306—18 (1930).

Stearn, A. E. Amphoteric Behaviour of Complex Systems: Theoretical. *J. Gen. Physiol.*, **10**, 313—23 (1926).

Svedberg, T., and Jette, E. Cataphoresis of Proteins. *J. Am. Chem. Soc.*, **45**, 954—7 (1923).

Theis, E. R. Industrial Biochemistry: Chemistry of the Proteins. *J. Am. Leather Chem. Assoc.*, **23**, 526—51 (1928).

Troensegaard, N., and Mygind, M. G. Cleavage Products from Hydrogenated Proteins. *Z. physiol. Chem.*, **193**, 171—80 (1930).

Weber, H. H., and Nachmannsohn, D. Independence of Protein Hydration and Ionisation. *Biochem. Z.*, **204**, 215—252 (1929).

Weber, H. H. Bjerrum's Theory of "Twin-Ions" and the Hydration of Proteins. *Ibid.*, **218**, 1—35 (1930).

Williams, R. R., and Waterman, R. E. Electrodialysis as a Means of Characterising Ampholytes. *Proc. Soc. Exptl. Biol. Med.*, **27**, 56—9 (1929).

MOLECULAR WEIGHT OF PROTEINS

Adair, G. S., and Robinson, M. E. The Analysis of the Osmotic Pressures of the Serum Proteins and the Molecular Weights of the Albumins and Globulins. *Biochem. J.*, **24**, 1864—89 (1930).

Cohn, E. J., Hendry, J. L., and Prentiss, A. M. The Minimal Molecular Weights of Certain Proteins. *J. Biol. Chem.*, **63**, 721—66 (1925).

Haas, A. R. C. Relative Masses of Protein Anions and Cations. *J. Phys. Chem.*, **22**, 520—4 (1918).

Horne, E. V. The Osmotic Pressure of Gelatin in Solutions of Sodium Salicylate. *Biochem. J.*, **18**, 1107—13 (1924).

Krishnamurti, K., and Svedberg, T. The Ultracentrifugal Study of Gelatin Solutions. *J. Am. Chem. Soc.*, **52**, 2897—2906 (1930).

Lamm, O. Theory and Methods of Ultracentrifuging. *Z. physik. Chem.*, **143**, 177—90 (1929).

Nichols, J. B. The Development of the Ultracentrifuge and its Field of Research. *Am. Coll. Symp. Monog.*, **6**, 287—308 (1928).

Noüy, P. L. D. Dimensions of Molecules and Molecular Weight of the Proteins of the Serum. *Compt. rend.*, **178**, 1904—6 (1924); Some New Aspects of the Surface Tension of Colloidal Solutions which have Led to the Determination of Molecular Dimensions. *Am. Coll. Symp. Monog.*, **3**, 25—47 (1925).

Sjögren, B., and Svedberg, T. The Molecular Weight of Legumin. *J. Am. Chem. Soc.*, **52**, 3279—83 (1930); The Molecular Weight of Lactalbumin. *Ibid.*, **52**, 3650—4 (1930).

Sjögren, B., and Spychalski, R. The Molecular Weight of Cocosin. *J. Am. Chem. Soc.*, **52**, 4400—4 (1930).

Stearn, A. E. The Determination of the Equivalent Weight of Proteins. *J. Gen. Physiol.*, **11**, 377—89 (1928).

Svedberg, T. Molecular Weight Estimation of Proteins by a

Centrifuge Method. Abderhalden's "Handbuch d. biol. Arbeitsmethoden," Abt. III. Teil B, S. 679—720 (1927); Mass and Size of Protein Molecules. *Nature*, **123**, 871 (1929); Ultracentrifugal Dispersion Determinations of Protein Solutions. *Kolloid-Z.*, **51**, 10—24 (1930).

Svedberg, T., and Fahraens, R. A New Method for the Determination of the Molecular Weight of the Proteins. *J. Am. Chem. Soc.*, **48**, 430—8 (1926).

Svedberg, T., and Sjögren, B. The pH-Stability Regions of Serum Albumin and of Serum Globulin. *Ibid.*, **52**, 2855—63 (1930). See also *Trans. Faraday Soc.*, **26**, 740—3 (1930).

Thomas, A. W., and Mayer, C. W. Estimation of the Acid-Combining Capacity of a Protein by Means of the Interferometer. *Proc. Soc. Exptl. Biol. Med.*, **25**, 667—9 (1928).

DENATURATION OF PROTEINS

Amar, J. Coagulation and Surface Tension. *Compt. rend.*, **178**, 522—5 (1924); Coagulation and Structure of the Egg. *Ibid.*, **178**, 803—5 (1924).

Anson, M. L., and Mirsky, A. E. The Reversibility of Protein Coagulation. *J. Phys. Chem.*, **35**, 185—93 (1931).

Bancroft, W. D., and Rutzler, J. E. The Denaturation of Albumin. *J. Phys. Chem.*, **35**, 144—61 (1931).

Beilinsson, A. Stabilisation of Protein Solutions to Heat with Sucrose and Glycerol. *Biochem. Z.*, **213**, 399—405 (1929).

Booth, N. The Denaturation of Proteins: The Titration of the Basic and Acidic Groups in Egg-Albumen. *Biochem. J.*, **24**, 158—68 (1930); The Denaturation of Proteins: Denaturation in the Presence of Alcohol. *Ibid.*, **24**, 1699—1705 (1930).

Buglia, G. On the Heat Coagulation of Liquid and Solid Organic Colloids. *Kolloid-Z.*, **5**, 291—3 (1909).

Chick, H., and Martin, C. J. The Heat-Coagulation of Proteins. *Kolloidchem. Beihefte*, **5**, 49—140 (1913/14).

Clark, J. H. Radiated Proteins: Coagulation of Egg-Albumin by Ultra-violet Light and Heat. *Am. J. Physiol.*, **73**, 647—60 (1925).

Cubin, H. K. Denaturation of Proteins: Denaturation by Acids. *Biochem. J.*, **23**, 25—30 (1929).

Huang, T., and Wu, H. Denaturation of Proteins: Osmotic Pressure of Denatured Egg Albumin and Methemoglobin in Concentrated Urea Solutions. *Chinese J. Physiol.*, **4**, 221—30 (1930).

Jochims, J. Connexion of Ropiness and Structure in Egg-White. *Biochem. Z.*, **203**, 142—46 (1928).

Lepeschkin, W. W. Denaturation of Albumins During Heat Coagulation. *Kolloid-Z.*, **31**, 342—5 (1922); Heat Coagulation of Proteins. *Biochem. Z.*, **16**, 678—701 (1922); The Coagulation of Denatured Protein. *Kolloid-Z.*, **32**, 168—73 (1923).

Lewis, P. S. The Kinetics of Protein Denaturation. Part I. The Effect of Variation in the Hydrogen Ion Concentration on the Velocity of the Heat Denaturation of Oxyhæmoglobin. *Biochem.*

J., **20,** 965—77 (1926); Part 2. The Effect of Variation in the Hydrogen Ion Concentration on the Velocity of the Heat Denaturation of Egg-Albumin; the Critical Increment of the Process. *Ibid.*, **20,** 978—83 (1926); Part 3. The Influence of Neutral Salts on the Velocity of the Heat Denaturation of Oxyhæmoglobin. *Ibid.*, **20,** 984—92 (1926); Part 4. The Free Basic and Acidic Groups of Fresh and Denatured Hæmoglobin. *Ibid.*, **21,** 46—53 (1927).

Lin, K., Wu, H., and Chen, T. Effect of Denaturation on Digestibility of Ovalbumin by Pepsin and Trypsin. *Proc. Soc. Exptl. Biol. Med.*, **25,** 199—200 (1927); *Chinese J. Physiol.*, **2,** 107—30 (1928).

Michaelis, L., and Rona, P. Extension of the Theory of Isoelectric Point. Competitive Action of Other Ions with Hydrogen and Hydroxyl Ions in the Precipitation of Denatured Albumins. *Biochem. Z.*, **94,** 225—39 (1919).

Ostwald, Wo. On the Influence of Electrolytes on the Temperature of Coagulation of Egg Albumin Solutions. *Kolloid-Z.*, **2,** 108—13, 138—42 (1907).

Peter, P. N., and Bell, R. W. Normal and Modified Foaming Properties of Whey-Proteins and Egg-Albumin Solutions. *Ind. Eng. Chem.* **22,** 1124—8 (1930).

Rajewsky, B. The Effect of Short-Wave Radiations on Proteins. *Biochem. Z.*, **227,** 272—85 (1930).

Rakusin, M. A., and Rosenfeld, A. The Coagulation of Albumin and *B*-Albumin. *Z. Untersuch. Lebensm.*, **49,** 38—9 (1925).

Ramsden, W. Separation of Solids in the Surface Layers of Solutions and Suspensions (Observations on Surface Membranes, Bubbles, Emulsions and Mechanical Coagulation). *Proc. Roy. Soc.*, **72,** 156—61 (1903); *Z. physik. Chem.*, **47,** 336—46 (1904); Surface Films, *Trans. Liv. Biol. Soc.*, **33,** 3—24 (1919); Some Physical Properties of Composite Surfaces. *Trans. Faraday Soc.*, **22,** 484—5 (1926).

Reiner, L., and Marton, A. Formaldehyde-Albumin. *Kolloid-Z.*, **32,** 273—9 (1923).

Sheppard, S. E., Hudson, J. H., and Houck, R. C. A Heat Coagulable Protein from Gelatin. *J. Am. Chem. Soc.*, **53,** 760—65 (1931).

Sørensen, M., and S. P. L. Coagulation of Proteins by Heating. *Compt. rend. trav. lab. Carlsberg*, **15,** (9), 1—26 (1925).

Spiegel-Adolf, M. Thermal Changes in Albumin. *Kolloid-Z.*, **38,** 127—9 (1926); *Biochem. Z.*, **170,** 126—72 (1926); Physico-Chemical Investigations on the Heat-Denaturisation of Proteins. Alexander's "Colloid Chemistry" Vol. 2, 303—26 (1928); Irradiated Proteins: Velocity of Coagulation by Light of Various Proteins. *Strahlentherap.*, **29,** 367—74 (1928); Protein Denaturation: Denaturation by Alcohol. *Biochem. Z.*, **204,** 1—13 (1929).

Tadokoro, T. Denaturation of Proteins. *J. Fac. Agric. Hokkaido*, **25,** 117—32, 133—49 (1928).

Wilheim, R. The Solution of Albumin Coagula by Neutral Salts. *Kolloid-Z.*, **48,** 217–31 (1929).

Waele, H. de. Gelation and Coagulation of Egg-White. *Ann. physiol. physicochim. biol.*, **5**, 514—8, 869—89 (1929).

Wu, H., and Yen, D. Studies on the Denaturation of Proteins. (1) Some New Observations Concerning the Effects of Dilute Acids and Alkalies on Proteins. *J. Biochem. Japan*, **4**, 345—84 (1924).

Wu, H., and Wu, D. Y. Nature of Heat Denaturation of Proteins *J. Biol. Chem.*, **64**, 369—78 (1925).

Wu, H. Studies on the Denaturation of Proteins. (2) Coagulation by Alcohol. *Chinese J. Physiol.*, **1**, 81—7 (1927); Denaturation Versus Coagulation. *Ibid.*, **3**, 1—6 (1929); A Theory of Denaturation and Coagulation of Proteins. *Am. J. Physiol.*, **90**, 562—3 (1929).

Wu, H., and Ling, S. M. Denaturation of Proteins: Factors Controlling Coagulation of Proteins by Shaking. *Chinese J. Physiol.*, **1**, 407—30 (1927).

Wu, H., and Tung-Ton, C. Effect of Denaturation and Coagulation on Acid- and Base-Binding Power of Proteins. *Ibid.*, **3**, 7—28 (1929); Liberation of Non-Protein Substances Upon Denaturation and Coagulation of Proteins. *Ibid.*, **3**, 75—80 (1929).

Young, E. G. Coagulation of Protein by Sunlight. *Proc. Roy. Soc.*, **B 93**, 235—48 (1922).

Young, E. G., and Macdonald, I. G. Peptic Digestion of Coagulated Egg-White. *Trans. Roy. Soc. Canada*, **21**, Sect. V., 385—93 (1927).

EGG-ALBUMIN

Akagi, S. The Relation of Viscosity of Some Protein Solutions to Temperature. *J. Biochem. Japan*, **11**, 423—5 (1930).

Cortese, D. Viscosity of Egg Albumin and its Variation in Fresh and Preserved Eggs. *Ann. chim. applicata*, **19**, 260—5 (1929).

Friese, W. The Proportion by Weight of Albumin, Yolk and Shell of Several Species of Fowl. *Z. Untersuch. Lebensm.*, **46**, 33—7 (1923).

Garreau, Y., and Marinesco, N. Dielectric Polarisation of Solutions of Egg Albumin. *Compt. rend.*, **189**, 331—3 (1929).

Ito, K. Combination of Egg Albumin with Halogen Ions. *J. Biochem. Japan*, **9**, 17—43 (1928).

Jochims, J. Relationship between Ability to be Spun and Structure of Egg-White. *Biochem. Z.*, **203**, 142—6 (1928).

John, J. L. St., and Green, E. L. Nature of Watery Whites in Eggs. Wash. Agr. Expt. Sta., *Bull.*, **237**, 1—22 (1930); The Colloidal Structure of Egg-White as Indicated by Plasticity Measurements. *J. Rheology*, **1**, 484—504 (1930).

Kinshiro, I. Biochemical Studies in the Equilibrium of the Halogen Ions: (1) On the Combination of Egg Albumin and the Halogen Ions. *J. Biochem. Japan*, **9**, 17—43 (1928).

Kreis, H., and Studinger, J. The Calcium Content of Egg-White. *Schweiz. Apoth.-Ztg.*, **59**, 193—6 (1921).

Marrack, J., and Hewitt, L. F. Osmotic Pressure of Egg Albumin. *J. Physiol.*, **66**, *Proc. Physiol. Soc.*, v (1928); Osmotic

Pressure of Crystalline Egg Albumin. *Biochem. J.*, **23**, 1079—89 (1929).

McClendon, J. F., and Prendergast, H. J. The Ultramicroscopy of Egg Albumin. *J. Biol. Chem.*, **38**, 549 (1919).

Nichols, J. B. The Molecular Weight of Egg Albumin : In the Presence of Electrolytes. *J. Am. Chem. Soc.*, **52**, 5176—87 (1930).

Ostwald, Wo., and Hertel, R. H. Colloid-Chemical Reactions Between Sols of Albumin and Polymeric Carbohydrates. *Kolloid-Z.*, **47**, 357—70 (1929).

Palmer, W. W., Atchley, D. W., and Loeb, R. F. Studies on the Regulation of Osmotic Pressure : the Effect of Increasing Concentrations of Albumin on the Conductivity of a NaCl Solution. *J. Gen. Physiol.*, **4**, 585—9 (1922).

Rakuzin, M. A., and Flier, G. D. Specific Gravity of Aqueous Solutions of Egg Albumin. *J. Russ. Phys. Chem. Soc.*, **48**, 458—61 (1916).

Schröder, V. Influence of Inorganic Ions on the Degree of Dispersion of Egg Albumin and Hæmoglobin at Various Hydrogen Ion Concentrations. *Biochem. Z.*, **195**, 210—9 (1928).

Scott, N. D., and Svedberg, T. Measurements of the Mobility of Egg Albumin at Different Acidities. *J. Am. Chem. Soc.*, **46**, 2700—7 (1924).

Sjögren, B., and Svedberg, T. The pH Stability of Egg Albumin. *Ibid.*, **52**, 5187—92 (1930).

Sørensen, S. P. L. Egg Albumin. *Tids. Kemi. Farm. Terapi*, **14**, 110—210 (1917).

Sørensen, S. P. L., and Høyrup, M. Preparation of Egg Albumin Solutions of Well-Defined Composition and the Analytical Methods Used. *Z. physiol. Chem.*, **103**, 15—89 (1918) ; *Compt. rend. trav. lab. Carlsberg*, **12**, 12—67 (1916) ; Composition and Properties of Egg Albumin Separated in Crystalline Form by Means of Ammonium Sulphate. *Ibid.*, **12**, 166—212 (1917) ; The State of Equilibrium between Crystallised Egg Albumin and Surrounding Mother Liquor and the Applicability of Gibbs' Phase Rule to Such Systems. *Ibid.*, **12**, 213—61 (1917).

Sørensen, S. P. L., Høyrup, M., Hempel, J., and Palitzsch, S. The Capacity of Acids and Bases to Combine with Egg Albumin. *Ibid.*, **12**, 68—163 (1917) ; *Z. physiol. Chem.*, **103**, 104—210 (1918) ; Osmotic Pressures of Egg Albumin Solutions. *Compt. rend. trav. lab. Carlsberg*, **12**, 262—372 (1917) ; *Z. physiol. Chem.*, **106**, 1—129 (1919).

Sørensen, S. P. L. The Egg Albumin of the Domestic Fowl. *Bull. soc. chim.*, **29**, 593—624 (1921).

Sørensen, S. P. L., Linderstrøm-Lang, K., and Lund, E. The Influence of Salts upon the Ionisation of Egg Albumin. *J. Gen. Physiol.*, **8**, 543—99 (1927).

Straub, J., and Hoogerduyn, M. J. J. Difference in Osmotic Concentration between Yolk and White of Egg. *Rec. trav. chim.*, **48**, 49—82 (1929).

Svedberg, T., and Nichols, J. B. The Molecular Weight of Egg

Albumin : In Electrolyte-Free Condition. *J. Am. Chem. Soc.*, **48**, 3081—92 (1926).

Takeda, M. Acid and Alkali Combinations with Protein. *J. Biochem. Japan*, **2**, 103—15 (1922).

Thomas, A. W., and Norris, E. R. The " Irregular Series " in the Precipitation of Albumin. *J. Am. Chem. Soc.*, **47**, 501—13 (1925).

Utzino, S. Dispersoidological Investigations on Proteins : I. The Temperature Effect on the Dispersion and Flocculation of Egg Albumin. *Kolloid.-Z.*, **47**, 244—8 (1929).

Vladimirov, G. E. Studies in Embryological Chemistry and Physiology : Certain Physico-Chemical Changes in the Egg-White of Developing Hen Eggs. *Biochem. Z.*, **177**, 280—97 (1926).

CASEIN

Buzágh, A. V. Wo. Ostwald's Solid-Phase Rule and the Solubility of Casein in Sodium Hydroxide. *Kolloid-Z.*, **49**, 185—88 (1929).

Clark, W. M. Studies in Technical Casein : (1) Introduction. *Ind. Eng. Chem.*, **12**, 1162—3 (1920).

Clark, W. M., Zoller, H. F., Dahlberg, A. O., and Weimar, A. C. Studies in Technical Casein : (2) Grain-Curd Casein. *Ibid.*, **12**, 1163—7 (1920).

Cohn, E. J. Studies in the Physical Chemistry of Protein : (1) The Solubility of Certain Proteins at Their Isoelectric Points. *J. Gen. Physiol.*, **4**, 697—722 (1922) ; (with **Hendry, J. L.**) (2) The Relation between the Solubility of Casein and its Capacity to Combine with Base. The Solubility of Casein in Systems containing the Protein and NaOH. *Ibid.*, **5**, 521—54 (1923) ; (with **Berggren, R. E.**) (3) The Relation between the Amino Acid Composition of Casein and its Capacity to Combine with Base. *Ibid.*, **7**, 45—79 (1924).

Fischer, M. H., and Hooker, M. O. Some Caseinates and the Theory of the Lyophilic Colloids. *Kolloid-Z.*, **47**, 193—206 (1929).

Gahl, R., and Greeves, R. L. Studies of Methods for the Measurement of the Electrical Conductivity of Protein Solutions with Special Reference to Solutions of Sodium Caseinate. *Univ. Calif. Publicns. Physiology*, **5**, 289—305 (1926).

Gahl, R., Greenberg, D. M., and Schmidt, C. L. A. Studies in the Formation and Ionisation of the Compounds of Casein with Alkali : Further Studies on the Conductivity of Casein Solutions. *Ibid.*, **5**, 307—13 (1926).

Greenberg, D. M., and Schmidt, C. L. A. Studies on the Formation and Ionisation of the Compounds of Casein with Alkali : (1) The Transport Numbers of Alkali Caseinate Solutions. *J. Gen. Physiol.*, **7**, 287—301 (1924) ; (2) The Conductivities of Alkali Caseinate Solutions. *Ibid.*, **7**, 303—16 (1924) ; (3) The Electrochemical Behaviour of Racemic Casein. *Ibid.*, **7**, 317—26 (1924) ; (4) The Transport Numbers of the Compounds of Casein with the Alkali Earth Elements. *Ibid.*, **8**, 271—8 (1926).

Kondo, K. Studies on Casein. *Compt. rend. trav. lab. Carlsberg*, **15**, 1—39 (1925).

Kruyt, H. R., and Lier, H. Lyophilic Colloids : Casein Sol. *Kolloidchem. Beihefte*, **28**, 407—50 (1929).

Kruyt, H. R., and Tendeloo, H. J. C. Charge, Hydration and Particle Size in Sols of Starch, Gum Arabic, Gelatin and Casein. *Ibid.*, **29**, 413—31 (1929).

Linderstrøm-Lang, K., and Kodama, S. Casein : (1) The Solubility of Casein in Hydrochloric Acid. *Compt. rend, trav. lab. Carlsberg*, **16**, 1—47 (1925) ; (2) Is Casein a Homogeneous Substance ? *Ibid.*, **16**, 48—62 (1925).

Linderstrøm-Lang, K. Casein (3) The Fractionation of Casein. *Ibid.*, **17**, No. 9, 1—116 (1929).

Loebenstein, F. Coagulation of Casein. *Kolloid-Z.*, **32**, 264—72 (1923) ; **34**, 227—30 (1924).

Moir, G. M. The Determination of the Milk Proteins : (1) The Chemistry of the Separation of Casein. *Analyst*, **56**, 2—9 (1931) ; (2) The Identity of the Casein Precipitate. *Ibid.*, **56**, 73—8 (1931) ; (3) Proposed Modified Method for Casein. *Ibid.*, **56**, 147—9 (1931).

Pauli, W. The Mobility of Protein Ions (Casein). *Biochem. Z.*, **127**, 150—5 (1922).

Pertzoff, V. The Effect of Temperature Upon Some of the Properties of Casein. *J. Gen. Physiol.*, **10**, 961—85 (1927) ; The Effect of Rennin Upon Casein : (1) The Solubility of Paracasein in NaOH. *Ibid.*, **10**, 987—1005 (1927) ; (2) Further Consideration of the Properties of Paracasein. *Ibid.*, **11**, 239—53 (1928).

Porcher, C. Action of Heat Upon the Complex, Calcium Caseinate + Calcium Phosphate. Greater Sensitivity of the Phosphate Micelles. *Compt. rend.*, **182**, 1247—9 (1926) ; Action of the Micelles of the Caseinate in the Complex Calcium Caseinate + Calcium Phosphate, its Consequences in the Action of Rennin on this Complex. *Ibid.*, **182**, 1420—2 (1926).

Rotonda, C. La. The Phenomenon of Coagulation. Influence of pH on the Separation of Casein by Electrolytes. *Ann. chim. applicata*, **19**, 310—28 (1929).

Schulz, M. E. Casein and Lactic Acid. *Milchwirtschaft. Forsch.*, **11**, 294—301 (1930).

Shaw, R. H. Studies of Technical Casein : Methods of Analysis. *Ind. Eng. Chem.*, **12**, 1168—70 (1920).

Slyke, L. L. V. A Summary of Research Studies Relating to Casein and Some of the Applications. New York State Agr. Expt. Sta. *Tech. Bull.*, **139** (1928).

Sørensen, S. P. L., and Sladek, I. Wo. Ostwald's Solid-Phase Rule and the Solubility of Casein in Sodium Hydroxide. *Compt. rend. trav. lab. Carlsberg*, **17**, No. 14, 1—35 (1929) ; *Kolloid-Z.*, **49**, 16—53 (1929).

Starlinger, W. Behaviour of Neutral Sodium Caseinate on Membrane Hydrolysis. *Biochem. Z.*, **170**, 1—17 (1926).

Svedberg, T., Carpenter, L. M., and Carpenter, D. C. The Molecular Weight of Casein. *J. Am. Chem. Soc.*, **52**, 241—52, 701—10 (1930).

Wright, N. C. Studies in Calcium-Caseinogen Equilibria and Their Bearing on the Secretion of Calcium in Milk (Preliminary Paper). *J. Agr. Sci.*, **16**, 640—2 (1926).

Zoller, H. F. Studies on Technical Casein : (4) Standardisation of the Borax Solubility Test for Commercial Caseins. *Ind. Eng. Chem.*, **12**, 1171—3 (1920) ; Casein Viscosity Studies. *J. Gen. Physiol.*, **3**, 635—51 (1921).

GELATIN

General

Abitz, W. *et al.* X-Ray Examination of Gelatin Micelles. *Naturwissenschaften*, **18**, 754—5 (1930).

Chevallier, A. Colloidal Micellar State of Gelatin. *Compt. rend. soc. biol.*, **97**, 484—5 (1927).

Elliott, F. A., and Sheppard, S. E. The Gold Number of Commercial Gelatins. *Ind. Eng. Chem.*, **13**, 699—700 (1921).

Frankel, M. The Colloid State of Gelatin Solutions and the Influence of Changes in Temperature Thereon. *Kolloid-Z.*, **45**, 355—66 (1928).

Gurian D. Technical Preparation of Glue and Gelatin. *Ibid.*, **54**, 106—12 (1931).

Gustavson, K. H. Specific Ion Effects in the Behaviour of Tanning Agents Toward Collagen Treated with Neutral Salts. *Am. Coll. Symp. Monog.*, **4**, 79—101 (1926).

Herrmann, K. *et al.* X-Ray Studies of the Structure of Gelatin Micelles. *Z. physik. Chem.*, **Abt. B., 10**, 371—94 (1930).

Knaggs, J., Manning, A. B., and Schryver, S. B. Investigations on Gelatin. Researches on the Methods of Purifying Gelatin. *Biochem. J.*, **17**, 473—87 (1923).

Knaggs, J., and Schryver, S. B. Purification of Gelatin by Flocculation in an Electric Field. *Ibid.*, **18**, 1079—84 (1924) ; Influence of the Treatment of the Precursor on the Character of the Gelatin. *Ibid.*, **18**, 1095—1101 (1924).

Kunitz, M., and Northrop, J. H. Fractionation of Gelatin. *J. Gen. Physiol.*, **12**, 379—390 (1929).

Kuntzel, A., and Buchheimer, K. Preparation and Properties of Raw Collagen. *Collegium* (**1930**), 205—217.

Lloyd, D. J. Notes on Some Properties of Dialysed Gelatin. *Biochem. J.*, **16**, 530—40 (1922).

Manning, A. B. The Properties of Gelatin Purified by Flocculation in an Electric Field. *Ibid.*, **18**, 1085—94 (1924).

Meunier, L. The Properties of Gelatin. *Chimie et industrie*, **5**, 642—50 (1921).

Nagorny, A. Ageing of Gelatin. *Kolloid-Z.*, **41**, 123—9 (1927).

Northrop, J. H., and Kunitz, M. Preparation of Electrolyte-Free Gelatin. *J. Gen. Physiol.*, **11**, 477—9 (1928) ; Combination of Salts and Proteins : III. The Combination of $CuCl_2$, $MgCl_2$, $CaCl_2$, $AlCl_3$, LaCl, KCl, $AgNO_3$, and Na_2SO_4 with Gelatin. *Ibid.*, **11**, 481—93 (1928).

Palmer, W. W. *et al.* Studies in the Regulation of Osmotic Pressure : The Effect of Increasing Concentrations of Gelatin on the Conductivity of NaCl Solution. *Ibid.*, **3**, 801—6 (1921).

Patten, H. E., and Kellems, T. O. Acidity of Ash-Free and of Commercial Gelatin Solutions. *J. Biol. Chem.*, **42**, 363—6 (1920).

Price, T. S. Descriptive Bibliography of Gelatin. Adhesives Research Committee, 1st Report, 51—126 (London, 1922) ; Gelatin, *Nature*, **110**, 286—8 (1923) ; *Science Progress*, **17**, 406—15 (1923).

Rakuzin, M. A. Fractionation of Gelatin by Alcohol, Aluminium Hydroxide and Barium Chloride. *Chem. Ztg.*, **48**, 249—50 (1924).

Rettig, F. Electrical Conductivity of Gelatin Mixtures and its Alteration with the Transformation of the Gelatin. *Kolloid-Z.*, **27**, 165—72 (1920).

Shukoff, I. I., and Bushmakin, I. N. Gelatin as an Emulsifying Agent. *J. Russ. Phys. Chem. Soc.*, **59**, 1061—9 (1927).

Thimann, K. V. The Effect of Salts on the Ionisation of Gelatin. *J. Gen. Physiol.*, **14**, 215—22 (1930).

Thimann, K. V., and Page, A. B. Chemical Change in Gelatin Resulting from the Method of Storage. *Biochem. J.*, **24**, 1058—60 (1930).

Wintgen, R., and Engelmann, H. Precipitation of Gelatin by Inorganic Colloids, *Kolloid-Z.*, **47**, 104—21 (1929).

Acid-Base Binding

Atkin, W. R., and Douglas, G. W. The Titration Curve of Gelatin. *J. Soc. Leather Trades' Chem.*, **8**, 359—70 (1924) ; *J. Am. Leather Chem. Assocn.*, **19**, 528—41 (1924).

Csapo, J. Influence of Neutral Salts on the Combination of Gelatin with Acids. *Biochem. Z.*, **159**, 53—7 (1925).

Ferguson, A. L., and Bacon, E. K. Diffusion-Potential Measurements Applied to HCl-Gelatin Systems. I. The Equivalent Weight of Gelatin. *J. Am. Chem. Soc.*, **49**, 1921—34 (1927) ; Diffusion Potential Measurements Applied to HCl-Gelatin Systems. II. The Components of HCl-Gelatin Solutions. *Ibid.*, **49**, 1934—9 (1927).

Ferguson, A. L. The Chemistry of the Body Processes : The Nature of the Action between Gelatin and Electrolytes. *Am. Coll. Symp. Monog.*, **5**, 159—78 (1928).

Higley, H. P., and Matthews, J. H. The Absorption Spectrum of Gelatin as a Function of the Hydrogen-Ion Concentration. *J. Am. Chem. Soc.*, **46**, 852—5 (1924).

Hitchcock, D. I. The Combination of Gelatin with HCl. *J. Gen. Physiol.*, **4**, 733—9 (1922) ; The Combination of Deaminised Gelatin. *Ibid.*, **6**, 95—104 (1923) ; Conductivity Titration of Gelatin Solutions with Acids. *Ibid.*, **6**, 201—5 (1923) ; The Combination of Gelatin with HCl : New Determinations of the Isoelectric Point and Combining Capacity of a Purified Gelatin. *Ibid.*, **12**, 495—509 (1929).

Lewis, W. K., and Daniell, C. F. Mass Action Effects in the Interaction of Gelatin and Acids. *Am. Coll. Symp. Monog.*, **4**, 122—31 (1926).

Lloyd, D. J., and Mayes, C. Titration Curve of Gelatin. *Proc. Roy. Soc.*, **B 93,** 69—85 (1922).

Loebel, Z. C. The Behaviour of Deaminised Gelatin. *J. Phys. Chem.*, **32,** 763—78 (1928).

Mathews, J. H., and Rowland, B. W. The Thermochemistry of Protein Behaviour. *Am. Coll. Symp. Monog.*, **1,** 227—31 (1923).

Petrumkin, A., and Petrumkin, M. Combination of Gelatin with Some Organic Bases. *J. Gen. Physiol.*, **11,** 101—10 (1927).

Isoelectric Point

Fallot, M. Magnetic Susceptibility and the Supposed Second Isoelectric Point of Gelatin. *Compt. rend.*, **186,** 1287—8 (1928).

Gerngross, O., and Bach, S. The Isoelectric Points of Glutin from Skin and Bones. *Biochem. Z.*, **143,** 542—52 (1923) ; The Isoelectric Points of Hide and Bone Gelatins. Collegium, (**1924**), 1—10 ; *J. Am. Leather Chem. Assocn.*, **19,** 262—5 (1924) ; Acid Action and Hydrogen Ion Concentration on Glue and Gelatin with Special Reference to Turbidity Phenomena at the Isoelectric Point and Jelly Formation. *Kolloid-Z.*, **40,** 279—86 (1926).

Hitchcock, D. I. The Isoelectric Point of Gelatin at 40° C. *J. Gen. Physiol.*, **6,** 457—62 (1924).

Johlin, J. M. The Isoelectric Point of Gelatin in Relation to its Physical Properties. *Proc. Soc. Exptl. Biol. Med.*, **26,** 702—4 (1929) ; The Isoelectric Point of Gelatin and its Relation to the Minimal Physical Properties of Gelatin. *J. Biol. Chem.*, **86,** 231—43 (1930).

Kraemer, E. O. The Second Isoelectric Point of Gelatin. *J. Phys. Chem.*, **29,** 410—3 (1925).

Rawling, S. O., and Clark, W. The Isoelectric Condition of Gelatin. *J. Chem. Soc.*, **121,** 2830—43 (1922).

Sheppard, S. E., and Houck, R. C. The Structure of Gelatin Sols and Gels : III. Isoelectric Points of Gelatin. *J. Phys. Chem.*, **34,** 2187—2201 (1930).

Stearn, A. E. The Nature of Isoelectric Gelatin in Solution. Evidence for the Existence of the Ampholyte Ion : (1) Ionic Displacements Reactions. *J. Phys. Chem.*, **34,** 971—80 (1930) ; (2) Conductivity Titrations of Gelatin with Crystal Violet. *Ibid.*, **34,** 981—92 (1930).

Wilson, J. A., and Kern, E. J. The Two Forms of Gelatin and Their Isoelectric Point. *J. Am. Chem. Soc.*, **44,** 2636—6 (1922).

Optical Behaviour

Bogue, R. H., and O'Connell, M. T. The Influence of Hydrogen Ion Concentration on the Optical Rotation of Gelatin. *J. Am. Chem. Soc.*, **47,** 1694—7 (1925).

Briefer, M. Photometric and Electrometric Measurements of Gelatin Behaviour. *Ind. Eng. Chem.*, **21,** 266—70 (1929).

Fanselow, J. R. The Influence of Electrolytes and Non-Electrolytes upon the Optical Activity and Relative Resistance to Shear of Gelatin Systems. *Am. Coll. Symp. Monog.*, **6,** 237—52 (1928).

Frankel, M. The Degree of Association and the Reactivity of Gelatin Solutions. *Z. physiol. Chem.*, **170**, 247—63 (1927).

Kraemer, E. O., and Fanselow, J. R. Studies of the Optical Activity of Gelatin Systems. *J. Phys. Chem.*, **29**, 1169—77 (1925); The Optical Activity and Colloidal Behaviour of Aqueous Gelatin Dispersions. *Ibid.*, **32**, 894—911 (1928).

Kraemer, E. O., and Dexter, S. T. The Light-Scattering Capacity (Tyndall Effect) and Colloidal Behaviour of Gelatin Sols and Gels. *Ibid.*, **31**, 764—82 (1927).

Mayer, K. Turbidity Phenomena in Gelatin. *Kolloid-Z.*, **44**, 315—9 (1928).

Ostwald, Wo. The Kinetics of Multirotation in Gelatin Sols. *Kolloid-Z.*, **17**, 113—9 (1915).

Smith, C. R. The Multirotation of Gelatin and its Significance in Gelation. *J. Am. Chem. Soc.*, **41**, 135—50 (1919).

Vles, F., and Vellinger. Rotatory Power of Gelatin. *Compt. rend.*, **180**, 439—42 (1925).

Surface Tension

Artom, C. The Surface Tension of Gelatin Solutions. *Boll. soc. biol. sper.*, **4**, 605—8 (1929); *Arch. sci., biol.*, **14**, 327—61 (1930).

Caro, L. de. The Surface Tension of Gelatin Solutions at Varying Hydrogen Ion Concentrations. *Atti. accad. Lincei*, **1**, 729—33 (1925).

Davis, C. E., Salisbury, H. M., and Harvey, M. T. Surface Tension of Gelatin Solutions. *Ind. Eng. Chem.*, **16**, 161—3 (1924).

Jermolenko, N. The Alteration of Surface Tension of Gelatin with the Alteration of pH and with Small Concentrations of Electrolytes. *Kolloid-Z.*, **48**, 141—6 (1929).

Johlin, J. M. The Influence of pH and Solution Concentration on the Surface Tension of Gelatin Solutions Determined by the Sessile-Drop Method. *J. Biol. Chem.*, **87**, 319—25 (1930).

Johnston, J. H. St., and Peard, G. T. Surface Tension of Gelatin Solutions. *Biochem. J.*, **19**, 281—9 (1925); **20**, 816—24 (1926).

Viscosity

Arrhenius, S. Viscosity and Hydration of Colloidal Solutions. *Medd. Vetenskapsakad. Nobelinst.*, **3**, (13), 1—20 (1916).

Bacon, E. K. Stoichiometric Relations in HCl-Gelatin Systems from Viscosity Measurements. *J. Phys. Chem.*, **33**, 1843—9 (1929).

Bogue, R. H. The Viscosity of Gelatin Sols. *J. Am. Chem. Soc.*, **43**, 1764—73 (1921).

Davis, C. E., Oakes, E. T., and Browne, H. H. The Viscosity of Gelatin Solutions. *J. Am. Chem. Soc.*, **43**, 1526—38 (1921).

Davis, C. E., and Oakes, E. T. Further Studies of the Physical Characteristics of Gelatin Solutions. *Ibid.*, **44**, 464—79 (1922).

Davis, C. E., and Salsbury, H. M. On the Prehistory of Gelatin in Relation to the Viscosity of its Solutions. *Kolloid-Z.*, **49**, 270—5 (1929).

Fodor, A., and Mayer, K. The Relation of Temperature to the Viscosity of Gelatin Solutions. *Kolloid-Z.*, **44**, 314—5 (1928).

Freundlich, H., and Neukircher, H. The Influence of Hydrogen Ion Concentration on the Viscosity and Elasticity of Gelatin Solutions. *Kolloid-Z.*, **38**, 180—1 (1926).

Fuseya, G., Sasaki, K., and Nagano, M. Viscosities of Dilute Solutions of Ash-Free Gelatin. *J. Phys. Chem.* **34**, 2012—27 (1930).

Gerngross, O., and Brecht, H. A. The Relation between Hydrolysis, Viscosity, Jelly Strength and Binding Strength in Glue and Gelatin. *Mitt. Materialprüfungsamt*, **40**, 253—67 (1922).

Shito, T. Physicochemical Study of Proteins. *Hokkaido J. Med.*, **7**, 187—215 (1929).

Shoji, R. Studies on Coagulation : The Viscosity of Gelation and Hydrolysis of Gelatin Sol. *Biochem. J.*, **13**, 227—38 (1919).

Membrane Equilibrium

Amy, L. Membrane Equilibria and Their Application in Biology. *J. pharm. chim.*, (8) **12**, 79—89 (1930).

Briggs, G. E., and Petrie A. H. K. On the Application of the Donnan Equilibrium to the Ionic Relations of Plant Tissues. *Biochem. J.*, **22**, 1071—82 (1928).

Donnan, F. G. The Theory of Membrane Equilibria. *Chem. Rev.*, **1**, 73—90 (1924) ; Thermodynamic Theory of Some Equilibria in Contact with Semipermeable Membranes. *Inst. intern. chimie Solvay*, 3me. conseil, 241—55 (1928) ; Theory of Ion Distribution Equilibria in a Gel System with Variable Micelle Distribution. *Kolloid-Z.*, **51**, 24—7 (1930).

Hill, A. V. Potential Difference Occurring in a Donnan Equilibrium and the Theory of Colloidal Behaviour. *Proc. Roy. Soc.*, **A 102**, 705—10 (1923) ; Membrane Potentials and Colloidal Behaviour. *J. Gen. Physiol.*, **6**, 91 (1923). See a reply to this by Hitchcock, *ibid.*, **6**, 93 (1923).

Hitchcock, D. I. Some Consequences of the Theory of Membrane Equilibria. *J. Gen. Physiol.*, **9**, 97—109 (1925).

Hückel, E. The Theory of Membrane Equilibrium. *Kolloid-Z.*, **36**, 204—17 (1925).

Northrop, J. H., and Kunitz, M. The Combination of Salts and Proteins : II. A Method for the Determination of the Concentration of Combined Ions from Membrane Potential Measurements. *J. Gen. Physiol.*, **9**, 351—60 (1926).

Procter, H. R., and Wilson, J. A. Acid-Gelatin Equilibrium. *J. Chem. Soc.*, **109**, 307—19 (1916).

Shiiji, K. Further Studies on the Donnan Theory of Membrane Equilibria. *Acta Scholae Med. Kyoto Imp. Univ.*, **10**, 325—32 (1928) ; Donnan's Membrane Equilibrium. *Kyoto J. Med.*, **26**, 57—67 (1929).

Wilson, J. A. The Swelling of Protein Jellies. *Am. Coll. Symp. Monog.*, **1**, 210—26 (1923).

Wintgen, R., and Vogel, H. The Equilibrium Gelatin-HCl. *Kolloid-Z.*, **30**, 45—53 (1922).

Wright, N. C. Membrane Equilibria and Selective Adsorption. *Biochem. J.*, **23**, 352—7 (1929).

Duclaux, J., and Titeica, R. Micellar Equilibria and Donnan Equilibria. *Rev. gén. coll.*, **8**, 251—9 (1930).

Swelling

Arisz, L. The Sol and Gel State of Gelatin Solutions : Gelatinising : Swelling. *Kolloidchem. Beihefte*, **7**, 1—90 (1915).

Bennett, H. G. The Swelling of Gelatin. *J. Am. Leather Chem. Assocn.*, **13**, 270—85 (1918). See the reply to this by Procter, *Ibid.*, **13**, 285—8 (1918).

Bogue, R. H. The Swelling and Gelation of Gelatin. *Ind. Eng. Chem.*, **14**, 32—5 (1922).

Brown, F. E. Density and Hydration in Gelatin Sols. *J. Am. Chem. Soc.*, **46**, 1207—9 (1924).

Buchner, E. H. Behaviour of Swollen Gelatin in Water Vapour. *Rec. trav. chim.*, **48**, 1047—51 (1929).

Efimov, V. V. Ion Migration in Gelatin Gels and in Nerve Substance : The Migration Velocity of H and OH ions of Different Concentrations under the Influence of an Electric Current. *Biochem. Z.*, **219**, 349—53 (1930) ; The Temperature Coefficient of the Migration Velocity of OH Ions in Gelatin Gels as Compared with the Temperature Coefficient at Different Phases of the Irritation Process in Nerve and Muscle. *Ibid.*, **219**, 361—3 (1930).

Ehrenberg R. Swelling of Gelatin in Aqueous Solutions. *Biochem. Z.*, **53**, 356—90 (1913).

Fairbrother, F., and Swan, E. The Dissolution of Gelatin. *J. Chem. Soc.*, **121**, 1237—44 (1922).

Fenn, W. O. Salt Antagonism in Gelatin. *Proc. Nat. Acad. Sci.*, **2**, 534—8 (1916).

Fischer, M. H. Hydration and " Solution " in Gelatin. *Kolloid-Z.*, **17**, 1—7 (1915) ; *Science*, **42**, 223—6 (1915).

Fischer, M. H., and Hooker, M. O. Swelling of Gelatin in Polybasic Acids and Their Salts. *J. Am. Chem. Soc.*, **40**, 272—92 (1918).

Fischer, M. H., and Coffman, W. D. Liquefaction or " Solution " of Gelatin in Polybasic Acids and Their Salts. *Ibid.*, **40**, 303—12 (1918).

Frankel, M. Thermal Disintegration of Gelatin. *Z. physiol. Chem.*, **167**, 17—36 (1927) ; The Colloid State of Gelatin and the Influence of Temperature Thereon. *Kolloid-Z.*, **45**, 355—66 (1928).

Gortner, R. A., and Hoffman, W. F. The Imbibition of Gelatin Dried as a Gel and as a Sol. *J. Phys. Chem.*, **31**, 464—6 (1927).

Herzog, R. O., and Weissenberg, K. Thermal, Mechanical and X-Ray Analysis of Swelling. *Kolloid-Z.*, **46**, 277—89 (1928).

Katz, J. R. The Laws of Swelling : I. The Swelling in Water Without Complication. *Kolloidchem. Beihefte*, **9**, 1—182 (1917).

Knaggs, J. Studies on Collagen. The Changes which Collagen Undergoes when Treated with Solutions of HCl and NaOH. *Biochem. J.*, **23**, 1308—27 (1929).

Krishnamurti, K. Mechanism of the Swelling of Gels. *Nature*, **123**, 242—3 (1929).

Kuhn, A. The Swelling of Gelatin in Aqueous Solutions of Organic Acids. *Kolloidchem. Beihefte*, **14**, 147—208 (1921).

Kunitz, M. Hydration of Gelatin in Solution. *J. Gen. Physiol.*, **10**, 811—36 (1927) ; Elasticity, Double Refraction and Swelling of Isoelectric Gelatin. *Ibid.*, **13**, 565—606 (1930).

Swelling of Gelatin in Aqueous Solutions of Acids, Bases and Salts and in Mixtures of These. *Biochem. Z.*, **209**, 326—437 (1929).

Labes, R., and Zain H. Antagonistic Action of Ions on Gelatin. *Arch. exptl. Path. Pharmakol.*, **146**, 63—77 (1929).

Lenk, E. Importance of Electrolytes for Swelling Processes : (1) The Action of the Individual Electrolytes. *Biochem. Z.*, **73**, 15—57 (1916) ; (2) Combinations of Electrolytes. *Ibid.*, **73**, 58—106 (1916).

Lloyd, D. J., and Pleass, W. B. Absorption of Water by Gelatin. *Biochem. J.*, **21**, 1352—67 (1926) ; Absorption of Water by Gelatin : (2) The Nitrate System. *Ibid.*, **22**, 1007—18 (1928).

Lloyd, D. J. Influence of Volume in Swelling. *Kolloid-Z.*, **48**, 342—45 (1929) ; On the Influence of Volume in Swelling. *Ibid.*, **54**, 46—9 (1931) ; Swelling of Gelatin in HCl and NaOH. *Biochem. J.*, **14**, 147—70 (1920) ; The Absorption of Water by Gelatin : The Influence of Temperature. *Ibid.*, **24**, 1460—71 (1930).

Miller, E. G. The Effect of Bicarbonate Ions on the Swelling of Gelatin. *Biochem. J.*, **23**, 876—9 (1929).

Moraczewski, W. v., and Hamerski, E. The Swelling of Gelatin. *Biochem. Z.*, **208**, 299—327 (1929).

Moraczewski, W. v., and Grzycki, S. The Swelling of Gelatin in Acids and Salts Solutions. *Ibid.*, **221**, 331—48 (1930).

Neville, H. A., and Theis, E. R. The Measurement of Hydration of Gelatin and Similar Materials and the Relation of Hydration to Swelling. *Am. Coll. Symp. Monog.*, **7**, 41—9 (1930). (With K'Burg) : A Study of Some Properties of Gelatin : Hydration of Gelatin and its Relation to Swelling. *Ind. Eng. Chem.*, **22**, 57—60 (1930).

Northrop, J. H., and Kunitz, M. The Swelling and Osmotic Pressure of Gelatin in Salt Solutions. *J. Gen. Physiol.*, **8**, 317—37 (1926) ; The Swelling Pressure of Gelatin and the Mechanism of Swelling in Water and Neutral Salt Solutions. *Ibid.*, **10**, 161—77 (1926) ; The Swelling of Gelatin and the Volume of Surrounding Solution. *Ibid.*, **12**, 537—42 (1929) ; Swelling and Hydration of Gelatin. *J. Phys. Chem.*, **35**, 162—84 (1931).

Northrop, J. H. The Swelling of Isoelectric Gelatin in Water : (1) Equilibrium Conditions. *J. Gen. Physiol.*, **10**, 893—903 (1927) ; (2) Kinetics. *Ibid.*, **10**, 905—26 (1927).

Ostwald, Wo., Kuhn, A., and Böhme, E. The Significance of the Hydrogen Ion Concentration for the Swelling of Gelatin. *Kolloidchem. Beihefte*, **20**, 412—33 (1925).

Ostwald, Wo. General Solvation Equation for Colloid Systems. *Kolloid-Z.*, **49**, 60—74 (1929).

Ostwald, Wo., and Kestenbaum, P. P. Relations of the Solid Phase in Swelling. *Kolloidchem. Beihefte*, **29**, 1—79 (1929).

Patten, H. E., and Johnson, A. J. The Effect of Hydrogen Ion Concentration on the Liquefaction of Gelatin. *J. Biol. Chem.*, **38**, 179—90 (1919).

Pleass, W. B. Absorption of Water by Gelatin : (3) The Sulphate System. *Biochem., J.*, **23**, 358—72 (1929) ; (5) The Influence of Calcium Hydroxide. *Ibid.*, **24**, 1472—8 (1930).

Popoff, M., and Seisoff, K. Agents Influencing Swelling of Gelatin. *Biochem. Z.*, **156**, 97—108 (1925).

Posnjak, E. Swelling Pressure. *Kolloidchem. Beihefte*, **3**, 417—56 (1912).

Procter, H. R. On the Action of Dilute Acids and Salt Solutions on Gelatin. *Kolloidchem. Beihefte*, **2**, 243—84 (1910/11).

Rudsit, K. The General Mass Action Relation for the Swelling of Gelatin in Electrolyte Solutions. *Kolloid-Z.*, **53**, 205—18 (1930).

Sauer, E., and Kleverkaus, E. The Swelling of Gelatin in Acids. *Kolloid-Z.*, **50**, 130—41 (1930).

Sheppard, S. E., and Elliott, F. A. The Drying and Swelling of Gelatin ; Preliminary Note. *J. Am. Chem. Soc.*, **44**, 373—9 (1922).

Shreve, E. B. Investigations on the Imbibition of Water by Gelatin. *Science*, **48**, 324—7 (1918) ; *J. Franklin Inst.*, **187**, 319—37 (1919).

Shukoff, I. I., and Shchukarev, S. A. Distribution of Hydrogen Ions between Gelatin and Water. *J. Phys. Chem.*, **29**, 285—94 (1925).

Simms, H. S. The Ionic Activity of Gelatin. *J. Gen. Physiol.*, **11**, 613—28 (1928) ; The Nature of the Ionizable Groups in Proteins. *Ibid.*, **11**, 629—40 (1928) ; Chemical Antagonism of Ions : Effect of Salt Mixture on Gelatin Activity, *Ibid.*, **12**, 511—28 (1929).

Smith, C. R. Osmosis and Swelling of Gelatin. *J. Am. Chem Soc.*, **43**, 1350—66 (1921).

Stiasny, E., and Gupta, S. D. R. Effect of Neutral Salts on Gelatin. (1) Swelling Action of Neutral Salts and the Value of the Hofmeister Series. *Collegium* (**1925**), 13—23 ; (2) Dispersive Effect of Neutral Salts. *Ibid.*, (1925), 23—36.

Svedberg, T., and Stein, B. A. Density and Hydration in Gelatin Solutions. *J. Am. Chem. Soc.*, **45**, 2613—20 (1923).

Terzaghi, C. The Mechanics of Adsorption and of the Swelling of Gels. *Am. Coll. Symp. Monog.*, **4**, 58—78 (1926).

Thiessen, P. A. Methods for the Quantitative Estimation of Swelling. *Kolloid-Z.*, **37**, 406—11 (1925).

Tolman, R. C., and Stearn, A. E. Molecular Mechanism of Colloidal Behaviour : The Swelling of Fibrin in Acids. *J. Am. Chem. Soc.*, **40**, 264—72 (1918).

Tsuda, S. Definite Temperatures in Gelatinising Systems. *Kolloid-Z.*, **47**, 28—36 (1929).

Wilson, J. A. The Swelling of Protein Jellies. *Am. Coll. Symp. Monog.*, **1**, 210—26 (1923).

Wilson, J. A., and Kern, E. J. Points of Minimum Swelling of Ash-Free Gelatin. *J. Am. Chem. Soc.*, **45**, 3139—40 (1923).

Gel Formation and Gel Structure.

Abramson, H. A. The Mechanism of the Inflammatory Process : Electrophoretic Migration of Inert Particles and Blood Cells in Gelatin Sols and Gels with Reference to Leucocyte Emigration Through the Capillary Wall. *J. Gen. Physiol.*, **11**, 743—65 (1928).

Aleev, A. E., and Gerasimov, A. F. Diffusion of NaCl and Na_2SO_4 in Concentrated Gelatin Gels. *J. Russ. Phys. Chem. Soc.*, **62**, 349—54 (1930).

Arisz, L. Sol and Gel Condition of Gelatin Solutions. *J. Am. Leather Chem. Assoc.*, **12**, 536—45 (1917).

Bachman, W. The Structure of Gels. *Kolloid-Z.*, **23**, 85—100 (1918).

Barratt, J. O. W. The Structure of Gels. The Physics and Chemistry of Colloids (Faraday Soc., London, 1921), 49—50.

Bogue, R. H. The Evaluation of Gelatin and Glue. *Ind. Eng. Chem.*, **14**, 435—41 (1922) ; The Sol-Gel Equilibrium in Protein Systems. *J. Am. Chem. Soc.*, **44**, 1313—22 (1922) ; The Structure of Elastic Gels. *Ibid.*, **44**, 1343—56 (1922) ; Plasticity and Structure in Gelatin Systems. *J. Phys. Chem.*, **29**, 1233—8 (1925).

Bradford, S. C. The Theory of Gels. *Biochem. J.* **12**, 351—81 (1918) ; The Reversible Sol-Gel Transformation. The Physics and Chemistry of Colloids (Faraday Soc., London, 1921), 44—8 ; The Nature of Gels. *Nature*, 111., 200—2 (1923) ; The Sol-Gel Transformation and the Properties of Jellies, Alexander's " Colloid Chemistry," Vol. I., 751—66 (New York, 1926).

Briefer, M., and Cohen, J. H. Pure Food Gelatin. Physical Properties Related to the Economy of Manufacture. *Ind. Eng. Chem.*, **20**, 408—13 (1928) ; Food Gelatin Values Relative to Concentration. *Ibid.*, **21**, 264—5 (1929).

Calábek, J., and Morávek, V. The Swelling of Gels Composed of Cholesterol, Lecithin and Gelatin. *Kolloid-Z.*, **50**, 141—7 (1930).

Free, E. E. Swelling of Gelatin and Agar Gels in Solutions of Sucrose and Dextrose. *Science*, **46**, 142—3 (1917).

Freundlich, H., and Seifriz, W. Elasticity of Sols and Gels. *Z. physik. Chem.*, **104**, 233—61 (1923).

Freundlich, H. Sol-Gel Transformations by Mechanical Working. *Kolloid-Z.*, **46**, 289—99 (1928) ; Structure of Colloidal Particles and Composition of Sols and Gels. *Ber.*, **61 B**, 2219—33 (1928).

Friedman, L. Diffusion of Non-Electrolytes in Gelatin Gels. *J. Am. Chem. Soc.*, **52**, 1305—10 (1930).

Friedman, L., and Kraemer, E. O. The Structure of Gelatin Gels from Studies of Diffusion. *Ibid.*, **52**, 1295—1304 (1930).

Gerngross, O., and Brecht, H. A. The Relation between Hydrolysis; Viscosity, Jelly-Strength and Binding-Strength in Glue and Gelatin. *Mitt. Materialprüfungsamt.*, **40**, 253—67 (1922) ; *Kolloid-Z.*, **33**, 353—4 (1923).

Gerngross, O. The Fine Structure of Gelatin Micelles. *Ledertech. Rundschau.*, **22**, 99—100 (1930).

Gortner, R. A., and Hoffman, W. F. Evidences of a Structure in Gelatin Gels. *Proc. Soc. Exptl. Biol. Med.*, **19**, 252—3, 257—64 (1922).

Hardy, W. B. Free and Bound water in Gels. *Kolloid-Z.*, **46**, 268—77 (1928).

Hatschek, E. An Analysis of the Theory of Gels as Systems of Two Liquid Phases. *Trans. Faraday Soc.*, **12**, 17—21 (1916) ; The Properties of Elastic Gels. Physics and Chemistry of Colloids (Faraday Soc., London, 1921), 35—9 ; Some Properties and Theories of Gels. *J. Soc. Chem. Ind.*, **48**, 389—99 (1929).

Hirota K. Studies on Coagulation : On the Empirical Equation for Gelation Velocity. *J. Biochem. Tokyo*, **9**, 103—15 (1928).

Kraemer, E. O. Structure of Gelatin Gels. *J. Phys. Chem.*, **29**, 1523—7 (1925) ; Observations of the Colloidal Behaviour of Aqueous Gelatin Systems. *Am. Coll. Symp. Monog.*, **4**, 102—21 (1926).

Kröger, M., and Fischer, K. Plastic Properties of Gels, Their Dependence on Temperature and the Formation of Gelatinous Lenses. *Kolloid-Z.*, **47**, 14—19 (1929).

Liepatov, I. S. The Kinetics of Swelling and Shrinkage of Gels. *Kolloid-Z.*, **36**, 222—6 (1925).

Lloyd, D. J. The Problem of Gel Structure. Alexander's. " Colloid Chemistry," Vol. I., 767—82 (New York, 1926).

Lottermoser, A., and Matthas, W. The Setting of Gelatin Sols. *Z. physik. Chem.*, **Abt. A. 141**, 129—36 (1929).

McBain, J. W. The Bearing of Results obtained in Recent Investigations of Soap Systems upon the Structure of Gelatin Gels. Adhesives Research Committee, 1st Report, 127—9 (London, 1922).

Michaud, F. The Rigidity of Jellies. *Ann. phys.*, **19**, 63—80 (1923).

Moeller, W. Internal and Surface Structure in Jellies. *Kolloid-Z.*, **18**, 213—9 (1916).

Neville, H. A., Theis, E. R., and Oswald, C. T. A Study of Some Properties of Gelatin : (2) A Method for Determining Transition Temperatures of Gels and Sols. *Ind. Eng. Chem.*, **22**, 60—2 (1930).

Oakes, E. T., and Davis, C. E. Jell Strength and Viscosity of Gelatin Solutions. *Ind. Eng. Chem.*, **14**, 706—10 (1922).

Ostwald, Wo. A Survey of the Field of Colloidal Gels. *Kolloid-Z.*, **46**, 248—67 (1928).

Pleass, W. B. Some Physical Conditions Affecting the Setting of Gelatin and the Bearing of the Results on the Theory of Gel Formation. *Proc. Roy. Soc.*, **A 126**, 406—26 (1930).

Poole, H. J. Elasticity of Gelatin Jellies and its Bearings on Their Physical Structure and Chemical Equilibrium. *Trans. Faraday Soc.*, **21**, 114—37 (1925).

Procter, H. R. The Structure of Elastic Jellies. Physics and Chemistry of Colloids (Faraday Soc., London, 1921), 40—3.

Pupilli, G. The Surface Tension of Protein Solutions at various pH Values Before and After Jellying. *Arch. sci. biol. Italy*, **12**, 373—92 (1928).

Reiger, R. The Kinetics of Gelatinisation and its General Significance. *Kolloidchem. Beihefte*, **19**, 381—440 (1924).

Rosinger, A., and Vetter, J. J. Method for Determining the Tensile Strength of Gelatin Jellies. *J. Am. Chem. Soc.*, **49**, 2994—3003 (1927).

Sauer, E., and Kinkel, E. The Determination of the Elasticity of Gelatin Gels. *Z. angew. Chem.*, **38**, 413—21 (1925).

Scarth, G. W. The Elasticity of Gelatin in Relation to pH and Swelling. *J. Phys. Chem.*, **29**, 1009—22 (1925).

Schaum, K. Some Processes and Conditions in Photographic Media and Other Gels. *Kolloid-Z.*, **36**, 199—203 (1925).

Serex, P., and Goodwin, M. W. Commercial Gelatins. Their Jelly Strength, Gold Number and Hydrogen Ion Concentration. *Ind. Eng. Chem.*, **19**, 473—4 (1927).

Sheppard, S. E., Sweet, S. S., and Scott, J. W. The Jelly Strength of Gelatins and Glues. *Ind. Eng. Chem.*, **12**, 1007—11 (1920).

Sheppard, S. E., and Sweet, S. S. The Setting and Melting Points of Gelatins. *Ibid.*, **13**, 423—6 (1921); The Elastic Properties of Gelatin Jellies. *J. Am. Chem. Soc.*, **43**, 539—47 (1921); Some Unresolved Factors in Jelly Strength Determination. *Ind. Eng. Chem.*, **16**, 593—5 (1924).

Sheppard, S. E., Sweet, S. S., and Benedict, A. J. Elasticity of Purified Gelatin Jellies as a Function of Hydrogen Ion Concentration. *J. Am. Chem. Soc.*, **44**, 1857—66 (1922).

Sheppard, S. E., and Houck, R. C. The Structure of Gelatin Sols and Gels. *J. Phys. Chem.*, **34**, 273—8 (1930).

Sheppard, S. E., and McNally, J. G. The Structure of Gelatin Sols and Gels. *Am. Coll. Symp. Monog.*, **7**, 17—39 (1930).

Shreve, E. B. Relation between Water Loss by Evaporation and Water Gain by Absorption in Colloidal Gels. Carnegie Inst. Washington, *Year Book*, **16**, 68—71 (1917).

Shoji, R. The Velocity of Gelation and Hydrolysis of Gelatin Sol. *Biochem. J.*, **13**, 227—38 (1919).

Smith, C. R. Determination of the Jellying Power of Gelatins and Glues by the Polariscope. *Ind. Eng. Chem.*, **12**, 878—81 (1920).

Stiles, W., and Adair, G. S. A Note on Diffusion in Gelatin Gels. *J. Am. Chem. Soc.*, **53**, 619—20 (1931).

Thompson, F. C. Structure of Gelatin Solutions. *J. Soc. Leather Trades' Chem.*, **3**, 209—16 (1919).

Tracey, A. F. A New Jelly Strength Tester and Some Experiments on Gelatin Gels. *J. Soc. Chem. Ind.*, **47**, 94—6 T. (1928).

Usher, F. L. A Mechanism of Gelatinisation. *Proc. Roy. Soc.*, **A 125**, 143—51 (1929).

Wagner, H. Consistency and Gel Formation. *Kolloid-Z.*, **47**, 19—21 (1929).

Weimarn, P. P. Von. The Jelly Condition of Matter, *Kolloid-Z.*, **36**, 175—6 (1925).

Yumikura, S. Osmosis of Several Acids in a Gelatin Gel. *Biochem. Z.*, **157**, 359—82 (1925).

Zsigmondy, R. Gel Structure. *Physik. Z.*, **14**, 1098—1105 (1913).

Syneresis.

Kuhn, A. Syneresis. *Kolloid-Z.*, **49**, 299—314 (1928).

Kunitz, M. Syneresis and Swelling of Gelatin. *J. Gen. Physiol.*, **12**, 289—312 (1928).

Liepatov. S. Viscosity and Hydration: (A) Ageing and Syneresis. *Kolloid-Z.*, **41**, 200—6 (1927); (B) The Kinetics of Syneresis. *Ibid.*, **43**, 396—40 (1927); (C) Fluids Separated by Syneresis and the Theory of Syneresis. *Biochem. Z.*, **192**, 91—104 (1928), and *J. Russ. Phys. Chem. Soc.*, **60**, 467—83 (1928); The Theory of Syneresis. *Kolloid-Z.*, **47**, 21—8 (1929); **48**, 62—3 (1929); **49**, 321—2 (1929); **49**, 441—50 (1929).

COLLOIDS IN CEREAL CHEMISTRY

Alsberg, C. L. The Colloid Chemistry of the Cereals. Bogue's "The Theory and Application of Colloidal Behaviour," Vol. 2, 581—604 (New York, 1924).

Bailey, C. H. A Biochemical Survey of Bread Production. *Am. Food J.*, **22**, 128—30 (1927).

Dearsley, W. L. M. Chemistry of Bread. Some New Zealand Wheat Flours and Fermentation. *N.Z. Jour. Sci. Tech.*, **8**, 34—8 (1925).

Elion, L. Biochemistry of Breadmaking. *Chem. Weekblad.*, **27**, 219—227 (1930).

Fisher, E. A. Field for Research in the Flour-Milling Industry. *Science Progress*, **19**, 614—27 (1925).

Gortner, R. A. Flour and Bread as Colloid Systems. *Ind. Eng. Chem.*, **15**, 1218—9 (1923).

Harrel, C. G. Some Variable Factors of Bread Production. *Cereal Chem.*, **3**, 1—18 (1925).

Mohs, K. Sizes of Pores in Baked Bread. *Cereal Chem.*, **1**, 149—51 (1924).

Swanson, C. O. A Theory of Colloid Behaviour in Dough. *Cereal Chem.*, **2**, 265—75 (1925).

Whymper, R. Colloid Problems in Bread Making. *Brit. Assoc. Coll. Reports*, **3**, 61—74 (1920).

STARCH

Alsberg, C. L. Studies upon Starch. *Ind. Eng. Chem.*, **18**, 190—3 (1926).

Arzichovski, W. Temperature of Swelling of Starch Granules. *Bull. acad. sci. russie.*, **1918** (6), 349—68.

Baldwin, M. E. Separation and Properties of the Two Main Components of Potato Starch. *J. Am. Chem. Soc.*, **52**, 2907—19 (1930).

Biedermann, W. Starch, Starch Granules and Starch Solutions. *Arch. ges. Physiol.* (*Pflüger's*), **183**, 168—96 (1920).

Broeze, J. R. Action of Ptyalin on Starch. *Biochem. Z.*, **204**, 286—302 (1929).

Chapman, O. W., and Buchanan, J. H. The Properties of Starch with Relation to Time of Formation of Starch Gels. *Iowa State Coll. J. Sci.*, **4**, 441—50 (1930).

Chodat, R. *et al.* The Specificity of Starches. *Compt. rend. des. séances soc. phys. hist. nat. Genèva*, **41**, 122—6 (1920).

Dox, A. W., and Roark, G. W. Determination of Gelatinisation Temperatures of Starches by Means of an Electrically Heated Chamber on the Microscope Stage. *J. Am. Chem. Soc.*, **39**, 742—5 (1917).

Fargher, R. G., and Probert, M. E. Examination of the Early Stages of the Hydrolysis of Starch by Dilute Acids. *J. Textile Inst.*, **18**, 559—72 T (1927).

Farrow, F. D., and Swan, E. Absorption of Water by Dried Films of Boiled Starch. *J. Textile Inst.*, **14**, 465—74 T (1923).

Francis, C. K., and Smith, C. C. Determination of the Gelatinising Temperature of the Starches from Grain Sorghums by Means of a Thermo-Slide. *Ind. Eng. Chem.*, **8**, 509—11 (1916).

Gorbachev, S. V. Formation of Paste from Starch. *J. Russ. Phys.-Chem. Soc.*, **62**, 355—64 (1930) ; *Biochem. Z.*, **224**, 91—101 (1930).

Gray, H. Le B., and Stand, C. J. Recent Advances in Cellulose and Starch Chemistry. *Chem. Reviews*, **4**, 355—73 (1927).

Harrison, W. Some Properties of Starch Considered from a Colloid-Chemical Standpoint. *J. Soc. Dyers Colourists*, **32**, 40—44 (1916).

Harvey, E. H. Some Physico-Chemical Properties of Starch. *Am. J. Pharm.*, **96**, 752—8, 816—22 (1924).

Herzfeld, E., and Klinger, R. Chemistry of the Polysaccharides. *Biochem. Z.*, **107**, 268—94 (1920).

Hess, K., Friese, H., and Smith, F. A. Potato Starch. *Ber.*, **61B**, 1975—82 (1928) ; **62B**, 1619—26 (1929).

Huss, H. The Swelling of Starch Grains. *Ark. för. Bot.*, **18**, 1—23 (1922).

Inukai, H. Soluble Starch. *Bull. Kyoto Ind. Research Inst.*, **1**, 1—23 (1927) **3**, 13—42 (1928).

Irvine, J. C., and Macdonald, J. Constitution of Polysaccharides : The Molecular Unit of Starch. *J. Chem. Soc.*, **128**, 1502—18 (1926).

Irvine, J. C. Chemistry of Starch. *Rec. trav. chim.*, **48**, 813—6 (1929).

Karrer, P. Constitution of Starch and Glycogen. *Naturwissenschaften*, **9**, 399—403 (1921).

Karrer, P., and Krauss, E. v. The Physical Structure of Starch. *Helv. Chim. Acta.*, **12**, 1144—52 (1929).

Katz, J. R. Is Starch Gelatinisation an Equilibrium Process Controlled by Mass Action ? *Verslag. Akad. Wettenschappen Amsterdam*, **23**, 1150—9 (1915).

Kuhn, R. Mechanism of the Action of Amylases : Constitution of Starch. *Ann.*, **443**, 1—71 (1925).

Lepeshkin, V. Studies on the Chemical Reactions during the Swelling of Starch in Hot Water. The Problem of High Tempera-

ture Coefficients. *Bull. soc. bot. Genèva*, **13**, 40—65 (1921) ; Swelling of Starch and Coagulation of Albumin by Heat. *Kolloid-Z.*, **32**, 42—4 (1923).

Ling, A. R., and Nanji, D. R. Starch : the Nature of Polymerised Amylose and of Amylopectin. *J. Chem. Soc.*, **123**, 2666—88 (1923) ; Starch : Constitution of Polymerised Amylose, Amylopectin and Their Derivatives. *Ibid.*, **127**, 629—36 (1925).

Ling, A. R. Recent Advances in Our Knowledge of Starch. *J. Soc. Chem. Ind.*, **46**, 279—81T (1927).

McLachlan, J. A. Some Aspects of the Manufacture of Starch and Glucose. *J. South Afr. Chem. Inst.*, **12**, 3—15 (1929).

Malfitano, G., and Catoire, M. Solubility and Insolubility of Starch. *Compt. rend.*, **177**, 1309—11 (1923) ; The Condition of the Micelles in Starch. *Kolloid-Z.*, **46**, 3—11 (1928).

Meyer, A. Contribution to the Knowledge of Jellies, Especially Starch Jellies. *Kolloidchem. Beihefte*, **5**, 1—48 (1913—14).

Meyer, K. H., Hopff, H., and Mark, H. Constitution of Starch. *Ber.*, **62B**, 1103—1112 (1929).

Náray-Szabó, St. v. Röntgen Diagram of Native Starch. *Ann.*, **465**, 299—304 (1928).

Peiser, E. The Constitution of Starch. *Z. physiol. Chem.*, **161**, 201—7 (1926) ; **167**, 88—90 (1927).

Philia, M. The Problem of the Constitution of Starch. *Bull. soc. botan. Genèva*, (2) **16**, 519—53 (1924).

Polak, F., and Tychowski, A. Chemistry of Starch. *Biochem. Z.*, **214**, 216—28 (1929).

Pringsheim, H., and Leibowitz, J. Chemistry of Starch : Constitution of the Polyamyloses. *Ber.*, **57B**, 884—7 (1924) ; Different Construction of the Two Constituents of Starch. *Ibid.*, **57B**, 887—91 (1924) ; Molecular Magnitude and Association of Polyamyloses. *Ibid.*, **59B**, 2058—64 (1926).

Pringsheim, H., and Meyersohn, P. The Constitution of Starch. *Z. physiol. Chem.*, **173**, 211—9 (1928).

Pringsheim, H., and Steingroever, A. Amylose and Amylopectin. *Ber.*, **62B**, 1352—9 (1929).

Pringsheim, H. Constitution of Starch and Cellulose. *Acta Chem. Fennica.*, **3**, 1—8 (1930).

Reychler, A. Starch. *Bull. soc. chim. Belg.*, **30**, 223—6 (1921) ; Starch Grains. *Ibid.*, **31**, 18—22 (1922).

Ritter, F. H. Nephelometric Studies on Starch Hydrosols : A Contribution to the Question of the Nephelometric Dilution Law. *Biochem. Z.*, **192**, 337—50 (1928).

Rosenthaler, L. Experiments on Starch Grains. *Schweiz. Apoth. Ztg.*, **61**, 654—9 (1923).

Sallinger, H. The Influence of Degree of Dispersity of Starch Solutions on the Phenomenon of So-called Starch Coagulation. *Kolloid-Z.*, **25**, 79—81 (1919).

Samec, M. Changes in the Colloidal State of Starch. *Intern. Z. physik.-chem. Biol.*, **1**, 173—88 (1914) ; Chemical Properties of Colloidal Starch Derivatives. *Compt. rend.*, **181**, 477—9 (1925) ;

Plant Colloids : Experiments on Wheat Starch. *Biochem. Z.*, **186,** 337—59 (1927) ; Plant Colloids : Experiments with Some Starch Dextrins. *Ibid.*, **187,** 120—36 (1927) ; " Die Kolloidchemie der Stärke " (Dresden , 1927) ; Plant Colloids : Behaviour of Starch Sols in the Dark Field. *Biochem. Z.*, **195,** 40—71 (1928) ; The Colloid Chemical Properties of Starch in Relation to Chemical Constitution. Walton's " A Comprehensive Survey of Starch Chemistry " (New York, 1928), pp. 51—61 ; The Condition of the Micelles in Starch. *Kolloid-Z.*, **47,** 81—2 (1929) ; Plant Colloids : Potato Amylopectin Prepared According to Different Methods. *Biochem. Z.*, **205,** 104—10 (1929).

Samec, M., and Haerdtl, H. Starches of Diverse Origin. *Kolloid-chem. Beihefte* **12,** 281—300 (1920).

Samec, M., and Tomazo, N. Solubility of Starch in Water Below the Swelling Temperature. *Ber.*, **62B,** 2076—78 (1929).

Samec, M. The Micellar Theory of Starch in its Present Form and the Physico-Chemical Behaviour of Starchy Substances. *Biochem. Z.*, **218,** 249—68 (1930).

Sherman, H. C., and Baker, J. C. Starch as a Substrate for Enzyme Action. *J. Am. Chem. Soc.*, **38,** 1885—1904 (1916).

Sponsler, O. L. Structural Units of Starch Determined by X-Ray Crystal Structure Method. *J. Gen. Physiol.*, **5,** 757—76 (1923).

Sprockhoff, M. Relation of the " Quality " of Potato Starch to the Formation of Paste. *Z. Spiritusind.*, **52,** 341—3, 350—1 (1929).

Tadokoro, T., and Sato, S. The Differences between Some Colloidal Properties of Common and Glutinous Rice Starch. *J. College Agr. Hokaido Imp. Univ.*, **12,** 1—65 (1923) ; **16,** 91—12[illegible] (1926).

Taylor, T. C., and Beckmann, C. O. The Disruption of the Corn Starch Granule and its Relation to the Constituent Amyloses. *J. Am. Chem. Soc.*, **51,** 294—302 (1929).

Taylor, T. C., and Walton, R. P. Characterisation of Certain Starches and Their Amyloses. *J. Am. Chem. Soc.*, **51,** 3421—40 (1929).

Thaler, H. Starch. *Mikrokosmos*, **22,** 174—8 (1928—9).

Weidenhagen, R. The Constitution of Starch. *Z. Ver. deut. Zuckerind.*, **77,** 474—80 (1927).

Williams, A. E. Manufacture of Rice Starch. *Ind. Chem.*, **6,** 387—9 (1930).

Windisch, W. Studies on Starch. *Wochschr. Brau.*, **41,** 3—5 (1924) ; **42,** 181—3, 187—9, 193—5, 199—201 (1925) ; Recent Progress in Our Knowledge of Starches. *Ibid.*, **44,** 369—72 (1927).

Wright, G. St. Vincent Arrowroot. *Trop. Agr.* (*Trinidad*), **5,** 162—6 (1928).

STARCH-IODIDE

Angelescu, E., and Mircescu, J. The Colour Produced by Iodine in the Presence of Starch. *J. phys. chim.*, **25,** 327—42 (1928).

Lottermoser, A., and Ott, A. Investigations on the Constitution of Iodine Starches. *Kolloid-Z.*, **52,** 138—59 (1930).

Staiger. The Reaction of Starch with Iodine. *Z. Spiritusind.*, **50,** 300 (1927).

Turner, R. G. The Starch-Iodide Reaction : Stability and Proportionality of Colour Produced by Small Amounts of Iodine. *J. Am. Chem. Soc.*, **52,** 2768—73 (1930).

Wu, H., and Wu, D. Y. Effect of Emulsoids on Coloured Solutions. Alexander's "Colloid Chemistry," Vol. I., 380—92 (New York, 1926).

FLOUR

Alsberg, C. L., and Griffing, E. P. Effect of Fine Grinding upon Flour. *Cereal Chem.*, **2,** 325—44 (1925).

Alsberg, C. L. Starch in Flour. *Cereal Chem.*, **4,** 485—92 (1927).

Bailey, C. H. A Method for the Determination of the Strength and Baking Qualities of Wheat Flour. *Ind. Eng. Chem.*, **8,** 53—7 (1916) ; Specific Conductivity of Water Extracts of Wheat Flour. *Science*, **47,** 645—7 (1918).

Bailey, C. H., and Collatz, F. A. Studies of Wheat Flour Grades : Electrical Conductivity of Water Extracts. *Science*, **51,** 374 (1920) ; *Ind. Eng. Chem.*, **13,** 319—21 (1921).

Bailey, C. H., and Weigley, M. Loss of CO_2 from Dough as an Index of Flour Strength. *Ind. Eng. Chem.*, **14,** 147—50 (1922).

Berczeller, L., and Wastl, H. The Chemical Composition of Wheat Kernels. *Biochem. Z.*, **177,** 168—80 (1926).

Berliner, E., and Koopmann, J. The Baking Power of Wheat Flours and Possibilities of its Determination. *Z. ges. Muhlenwesen*, **4,** 119—25 (1927).

Blish, M. J., and Sandstedt, R. M. Factors Influencing Interpretation of Experimental Baking Tests. *Cereal Chem.*, **4,** 291—9 (1927).

Blish, M. J. Standard Experimental Baking Test. *Ibid.*, **5,** 158—61 (1928). Report of Committee on Standardisation of Experimental Baking Test. *Ibid.*, **5,** 277—300 (1928).

Brownlee, W. E., and Bailey, C. H. Proteolysis in Bread Doughs. *Ibid.*, **7,** 487—517 (1930).

Buchanan, J. H., and Naudain, G. G. Influence of Starch on Strength of Wheat Flour. *Ind. Eng. Chem.*, **15,** 1050—1 (1923).

Cazaubon, E. Some Properties of Wheat Flours. *Bull. assoc. chim. sucr., dist.*, **47,** 44—5 (1930).

Coleman, D. A. *et al.* Milling and Baking Qualities of World Wheats. U.S. Dept. Agric., *Tech. Bull.*, **197,** 1—224 (1930).

Collatz, F. A. Flour Strength as Influenced by the Addition of Diastatic Ferments. *Am. Inst. Baking, Bull.*, **9,** 1—73 (1922).

Engledow, F. L. An Aid in the Determination of Flour Strength. *Cereal Chem.*, **5,** 1—9 (1928).

Fellenberg, T. v. Experiments on the Baking Properties of Flour. *Mitt. Lebensm. Hyg.*, **10,** 229—60 (1919).

Foster, L. D. Testing of New Zealand Grown Wheats : Baking Tests. *N. Zealand J. Agr.*, **32,** 26—33 (1926) ; Quality of the Flours. *Ibid.*, **32,** 93—104 (1926).

Geddes, W. F. Chemical and Physico-Chemical Changes Induced in Wheat and Wheat Products by Elevated Temperatures. *Can. J. Res.*, **2,** 65—90 (1930).

Haas, L. W. Interpretation of Baking Tests. *Cereal Chem.*, **4,** 389—94 (1927).

Herman, R. S., and Hart, V. M. Some Factors Influencing the Experimental Baking Test. *Cereal Chem.*, **4,** 157—83 (1927).

Kalning H., and Schleimer, A. Modification of the Constituents of Flour which Occurs During Baking. Chemical Composition of Bread. *Z. ges. Getreidew.*, **6,** 137—43 (1914).

Karacsonyi, L. P., and Bailey, C. H. Relation of the Overgrinding of Flour to Dough Fermentation. *Cereal Chem.*, **7,** 571—86 (1930) ; Effect of Overgrinding of Flour on the Keeping Quality of Bread. *Ibid.*, **8,** 44—46 (1931).

Lee, G. van der. The Significance of the Fineness of Flour for its Use in Bakeries. *Chem. Weekblad.*, **25,** 34—40 (1928).

Martin, F. J. Properties Affecting Strength in Wheaten Flour. *J. Soc. Chem. Ind.*, **39,** 246—51 T (1920).

Molin, G. The Milling Value of Wheat. *Kgl. Landtbruks-Akad. Handl. Tid.*, **64,** 231—8 (1925).

Olson, G. A. Wheat and Flour Investigations. Washington Agr. Expt. Sta., *Bull.*, **144,** 5—86 (1917).

Ostwald, Wa., and Riedel, A. The Preparation of Bread from Starch. *Kolloid-Z.*, **17,** 12—4 (1915).

Remington, J. S. The Conditioning of Wheat. *Ind. Chem.*, **2,** 203—7 (1926).

Rumsey, L. A., Diastatic Enzymes of Wheat Flour and Their Relation to Flour Strength. *Am. Inst. Baking, Bull.*, **8,** 1—85 (1922).

Schollenberger, J. H., and Colman, D. A. Influence of Granulation on Chemical Composition and Baking Quality of Flour. U.S. Dept. Agr., *Bull.*, **1463,** 1—35 (1926).

Settimj, L. The Different Chemical Components of the Crust and of the Crumb of Wheat Bread of Rome. *Ann. chim. applicata.*, **18,** 19—31 (1928).

Stockham, W. L. The Capacity of Wheat and Mill Products for Moisture. N. Dakota Agr. Expt. Sta., *Bull.*, **120,** 97—131 (1917).

Swanson, C. O. *et al.* Kansas Flours : Chemical, Baking and Storage Tests. Kansas Agr. Expt. Sta., *Bull.*, **202,** 1—135 (1915) ; The Mechanical Method of Modification of Dough. *Cereal Chem.*, **5,** 375—85 (1928). Factors of Flour Quality : The Colloid Particle. *Miller's Rev.*, **92,** (5), 27—9 (1928).

Weaver, H. A., and Goldtrap, W. A. Flour Strength. *J. Am. Assoc. Cereal Chem.*, **7,** 115—23 (1922).

Willard, J. T., and Swanson, C. O. The Influence of Certain Substances upon the Baking Qualities of Flours. Kansas Agr. Expt. Sta., *Bull.*, **190,** 237—85 (1914).

Ziegelmayer, W. The Action of Plant Lecithin on the Swelling and Surface Tension of the Gluten and Starch of Flour : Contribution to the Investigation of Binding in Baking. *Kolloid-Z.*, **53**, 224—31 (1930).

Zinn, J. Correlations between Various Characters of Wheat and Flour as Determined from Published Data from Chemical, Milling and Baking Tests of a Number of American Wheats. *J. Agr. Research*, **23**, 529—48 (1923).

ACIDITY RELATIONS OF FLOURS

Arbouet, B. d'. The Bread-Making Value of Wheat Flour. *Chimie et industrie*, **14**, 21—8 (1925) ; Note on Bread-Making. *Rev. gén. coll.*, **4**, 199—201, 225—9 (1926).

Bailey, C. H., and Peterson, A. C. Wheat Flour Grades : Buffer Action of Water Extracts. *Ind. Eng. Chem.*, **13**, 916—8 (1921).

Bailey, C. H. Report on the Hydrogen Ion Concentration of Flour. *J. Assoc. Official Agr. Chem.*, **10**, 469—73 (1927).

Britton, H. T. S. The Importance of Hydrogen Ion Concentration in Baking. *Ind. Chem.*, **5**, 160—3 (1929).

Clark, R. J. Bread Troubles in the Light of Hydrogen Ion Concentration. *Cereal Chem.*, **1**, 161—7 (1924).

Cohen, E. J., and Henderson, L. J. Physical Chemistry of Bread Making. *Science*, **48**, 501—5 (1918).

Fisher, E. A., and Halton, P. Relation of Hydrogen Ion Concentration and Buffer Value to the Baking Quality of Flour. *Cereal Chem.*, **6**, 18—32, 97—115 (1929).

Foster, L. D. Chemistry of New Zealand Wheats and Flours : Degree of Buffering and Baking Value of Some Local Wheat Flours. *N. Zealand J. Sci. Tech.*, **8**, 236—42 (1926).

Grewe, E., and Bailey, C. H. Relation of Hydrogen Ion Concentration of Dough to Baking Properties. *Cereal Chem.*, **4**, 261—70 (1927).

Halton, P., and Fisher, E. A. Determination of Hydrogen Ion Concentration of Flour-Water Mixtures. *Cereal Chem.*, **5**, 445—60 (1928).

Harrel, C. G. Value of Hydrogen Ion Concentration and Buffer Value Determination in Testing and Use of Flours. *Cereal Chem.*, **4**, 423—35 (1927).

Jessen-Hansen, H. Studies on Wheat Flour : Influences of Hydrogen Ion Concentration on Baking Value of Flour. *Compt. rend.*, **10**, 170—206 (1911).

Markley, M. C. Certain Effects of Varying the Hydrogen Ion Concentration of the Tempering Water upon the Wheat Proteins. *Cereal Chem.*, **7**, 154—61 (1930).

Sørensen, S. P. L. Hydrogen Ion Concentration in Bread-Making. *Am. Food J.*, **19**, 556—60 (1924).

VISCOMETRY OF FLOUR

Amar, J. Coagulation and Vegetable Life. *Compt. rend.*, **178**, 1317—9 (1924).

Bailey, C. H., and Vesconte, A. M. Le. Physical Tests of Flour Quality with the Chopin Extensimeter. *Cereal Chem.*, **1**, 38—63 (1924).

Biechy, T., Muller, R. *et al.* The Alkali-Viscosity of Pure Wheat Flours and its Relation to Practical Bread-Making. *Kolloid-Z.*, **54**, 194—210 (1931).

Blair, G. W. S., Watts, G., and Denham, H. J. Effect of Concentration on Viscosity of Flour Suspensions. *Cereal Chem.*, **4**, 63—7 (1927).

Blish, M. J., and Sandstedt, R. M. Viscosity Studies with Nebraska Wheat Flours. *Cereal Chem.*, **2**, 191—201 (1925).

Chopin, M. Relation between the Mechanical Properties of Dough and Bread-Making. *Bull. soc. encour. ind. nat.*, **133**, 261—73 (1921) ; Determination of Baking Value of Wheat by Measure of Specific Energy of Deformation of Dough. *Cereal Chem.*, **4**, 1—13 (1927).

Denham, H. J., Blair, G. W. S., and Watts, G. Notes on the Use of Ostwald Viscometers for Flour Suspensions. *Cereal Chem.*, **4**, 206—220 (1927).

Dunn, J. A. Plasticity—its Possibilities in Cereal Research. *Cereal Chem.*, **3**, 351—9 (1926).

Earlenbaugh, L. Effect of Mono-Calcium Phosphate upon the Viscosity of Acidulated Flour-in-Water Suspensions. *Cereal Chem.*, **3**, 102—6 (1926).

Farrow, F. D., and Lowe, G. M. Flow of Starch Paste Through Capillary Tubes. *J. Textile Inst.*, **14**, 414—40 T (1923).

Freundlich, H., and Nitze, H. The Viscosity and Elasticity of Starch Paste. *Kolloid-Z.*, **41**, 206—15 (1927).

Gortner, R. A., and Sharp, P. F. Physicochemical Properties of Strong and Weak Flours : Viscosity as a Measure of Hydration Capacity and the Relation of the Hydrogen Ion Concentration to Imbibition in the Different Acids. *J. Phys. Chem.*, **27**, 481—92 (1923) The Influence of Ash of Flours upon the Viscosity of Flour-in-Water Suspensions. *Ibid.*, **27**, 567—76 (1923).

Gortner, R. A. Viscosity as a Measure of Gluten Quality. *Cereal Chem.*, **1**, 75—81 (1924).

Harrel, C. G. A Gravimetric Penetrometer for Measuring Stiffness or Toughness of Doughs. *Cereal Chem.*, **4**, 283—90 (1927).

Heiduschka, A., and Fichte, E. Contribution to the Understanding of the Baking Power of Flours. *Kolloid-Z.*, **32**, 193—5 (1923).

Henderson, L. J., Fenn, W. O., and Cohn, E. J. Influence of Electrolytes upon the Viscosity of Dough. *J. Gen. Physiol.*, **1**, 387—97 (1919).

Hermans, A. J., and Rask, O. S. A Consideration of Certain Reactions of Starches with Special Reference to Enzyme Hydrolysis. *Cereal Chem.*, **3**, 361—92 (1926).

Herschel, W. H., and Bergquist, C. The Consistency of Starch and Dextrin Pastes. *Ind. Eng. Chem.*, **13**, 703—6 (1921).

John, J. L. St. Plasticity of Flour-Water Suspensions. *Cereal Chem.*, **6**, 400—10 (1929).

Johnson, A. H., and Bailey, C. H. A Physico-Chemical Study of Cracker Dough Fermentation. *Cereal Chem.*, **1**, 327—410 (1924).

Johnson, A. H. Wheat and Flour Studies : Factors Influencing the Viscosity of Flour-Water Suspensions : (A) Effects of Temperature, Degree of Hydration and Method of Manipulation. *Cereal Chem.*, **4**, 87—128 (1927) ; (in collaboration with **Herrington, B. L.**) ; (B) Effect of Hydrogen Ion Concentration During Extraction or Digestion Period. *Ibid.*, **5**, 14—44 (1928) ; (C) Effect of Small Quantities of CO_2 in Water Used for the Extraction of Electrolytes. *Ibid.*, **5**, 105—116 (1928).

Jong, H. G. B. de. The Electroviscous Effect in Solutions of Soluble Starch. *Rec. trav. chim.*, **43**, 190—202 (1924).

Karacsonyi, L. P. A Quick Viscosimetric Method for Measuring the Staleness of Bread. *Cereal Chem.*, **6**, 241—3 (1929).

Lee, G. van der. The Viscosity of Flour Suspensions. *Cereal Chem.*, **5**, 10—3 (1928).

Lüers, H., and Ostwald, Wo. Colloid Chemistry of Bread : Viscometry of Flour. *Kolloid-Z.*, **25**, 82—90, 116—36 (1919).

Lüers, H., and Schwarz, M. Relation of Viscosity to the Baking Quality of Flours. *Z. Untersuch. Lebensm.*, **49**, 75—89 (1925).

Mohs, K. Viscosity and Baking Ability. *Chem.-Ztg.*, **47**, 889—91 (1923).

Morgan, R. W. A Practical Application of the Viscometer to the Mill. *Cereal Chem.*, **1**, 288—92 (1924).

Nowopokrowsky, L., and Tschebotarewa, N. The Gelatinisation of Potato Starch and its Colloid-Chemical Properties. *Kolloid-Z.* **52**, 302—4 (1930).

Porst, C. E. G., and Moskowitz, M. Comparison of the Various Corn-Products Starches as shown by the Bingham-Greene Plastometer. *Ind. Eng. Chem.*, **14**, 49—52 (1922).

Rask, O. S., and Alsberg, C. L. A Viscosimetric Study of Wheat Starches. *Cereal Chem.*, **1**, 7—26 (1924).

Schulz and Parlow. Influence of Low Temperatures on the Viscosity of Potato Starch. *Z. Spiritusind.*, **53**, 135—6 (1930).

Sharp, P. F. Plasticity of Simple Flour-in-Water Suspensions. *Cereal Chem.*, **3**, 40—55 (1926).

Sharp, P. F., and Gortner, R. A. Viscosity as a Measure of Hydration Capacity of Wheat Flour and its Relation to Baking Strength. Univ. Minn. Agr. Expt. Sta., *Bull.*, **19**, 1—119 (1923) ; Physicochemical Properties of Strong and Weak Flours : Relation Between the Maximum Viscosity Obtainable by the Addition of Lactic Acid and the Concentration of Flour-in-Water Suspensions. *J. Phys. Chem.*, **27**, 771—88 (1923) ; (in collaboration with **Johnson, A. H.**) Physical State of the Gluten as Influencing the Loaf Volume. *Ibid.*, **27**, 942—7 (1923).

Smith, E. E. Viscosity and Baking Quality. *Cereal Chem.*, **2**, 177—90 (1925).

Yoshino, M. Starch Paste : Relation Between the Viscosity of Wheat Starch Paste and the Concentration and the Temperature. *J. Chem. Ind.* (*Japan*), **26**, 870—3 (1923).

PROTEINS OF FLOUR

Alsberg, C. L., and Griffing, E. P. The Heat Coagulation of Gluten. *Cereal Chem.*, **4**, 411—23 (1927).

Andre, H. d'. Importance of the Value of the "Absorptive Capacity" of the True Gluten and its Practical Use in Flour Analysis. *J. Am. Assoc. Cereal Chem.*, **7**, 30—2 (1922).

Arbouet, B. d'. Notes on Gluten. *Rev. gén. coll.*, **4**, 97—107 (1926).

Bailey, C. H., and Blish, M. J. The Identity of the Proteins Extracted from Wheat Flour by the Usual Solvent. *J. Biol. Chem.*, **23**, 345—57 (1915).

Bailey, C. H., and Sherwood, R. C. Relation of the Crude Protein Content of Flour to Loaf Volume. *Cereal Chem.*, **3**, 393—401 (1926).

Blish, M. J. On the Chemical Constitution of the Proteins of Wheat Flour and its Relation to Baking Strength. *Ind. Eng. Chem.*, **8**, 138—44 (1916).

Blish, M. J., and Pickney, A. J. The Identity of Gluten Proteins from Various Wheat Flours. *Cereal Chem.*, **1**, 309—16 (1924).

Blish, M. J. The Individuality of Glutenin. *Ibid.*, **2**, 127—31 (1925).

Blish, M. J., and Sandstedt, R. M. Glutenin : A Simple Method for its Preparation and Direct Determination. *Ibid.*, **2**, 57—67 (1925). An Improved Method for the Preparation of Wheat Gliadin. *Ibid.*, **3**, 144 (1926).

Blish, M. J. Report on Glutenin in Wheat Flour. *J. Assoc. Official Agr. Chem.*, **9**, 417—23 (1926) ; The Quantitative Estimation of Glutenin in Wheat Flour. *Cereal Chem.*, **4**, 129—35 (1927) ; The Gluten and Non-Proteins. *Ibid.*, **7**, 421—7 (1930).

Chamberlain, J. S. Properties of Wheat Proteins. *J. Am. Chem. Soc.*, **28**, 1657—67 (1906).

Coleman, D. A. *et al.* A Comparison of Some Physical and Chemical Tests for Determining the Quality of Gluten in Wheat and Flour. *J. Agr. Research*, **34**, 241—64 (1927).

Csonka, F. A., and Jones, D. B. Studies on Glutelins : The α and β-Glutelins of Wheat (*Triticum vulgare*). *J. Biol. Chem.*, **73**, 321—9 (1927).

Dupont, A. The Variation in the Nitrogen and Gluten Content of Wheat. *Ann. sci. agron.*, **42**, 256—75 (1925).

Fisher, E. A. A Study of the Rate of Drying of Wheat Flour, Starch and Gluten. *Cereal Chem.*, **4**, 184—206 (1927).

Foster, L. D. Chemistry of New Zealand Wheats and Flours : Hydration Capacity of Gluten from Some Local Samples. *N. Zealand J. Sci. Tech.*, **8**, 299—304 (1926).

Geddes, W. F., and Goulden, C. H. A Study of the Utility of Protein Peptisation by Inorganic Salt Solutions as a Means of Predicting Loaf Volume. *Cereal Chem.*, **7**, 527—55 (1930).

Gericke, W. F. Variation in the Percentage of Protein in the Grain of a Single Wheat Plant. *Science*, **71**, 73—4 (1930).

Gerum, J., and Metzer, C. Wheat Gluten. *Z. Untersuch. Lebensm.*, **46**, 74—86 (1923).

Gortner, R. A., and Doherty, E. H. Hydration Capacity of Gluten from "Strong" and "Weak" Flours. *J. Agr. Research*, **13**, 389—419 (1918).

Gortner, R. A., Hoffman, W. F., and Sinclair, W. B. The Peptization of Wheat Flour Proteins by Inorganic Salt Solutions. *Cereal Chem.*, **6**, 1—17 (1929).

Grewe, E., and Bailey, C. H. The Concentration of Glutenin and Other Proteins in Various Types of Wheat Flour. *Cereal Chem.*, **4**, 230—47 (1927).

Groh, J., and Friedl, G. Physico-Chemical Properties of the Alcohol-Soluble Proteins of Wheat and Rye. *Biochem. Z.*, **66**, 154—64 (1914).

Guess, H. A. The Gluten Constituents of Wheat and Flour and Their Relation to Breadmaking Qualities. *J. Am. Chem. Soc.*, **22**, 263—8 (1900).

Hall, H. A Comparative Study of the Non-Gluten Constituents of Hard and Soft Wheat Flours. *Cereal Chem.*, **7**, 270—9 (1930).

Halton, P. The Chemistry of the Strength of Wheat Flour. *J. Agr. Research*, **14**, 587—99 (1924).

Harris, R. H. Relation between Crude Protein Content and Loaf Volumes obtained by Two Different Methods of Baking. *Cereal Chem.*, **7**, 557—70 (1930); Relation of Peptization of Wheat Flour Protein to Loaf Volume. *Ibid.*, **8**, 47—62 (1931).

Henderson, L. J. *et al.* A Study of the Action of Acid and Alkali on Gluten. *J. Gen. Physiol.*, **1**, 459—72 (1919).

Herd, C. W. A Study of Some Methods of Examining Flour, with Special Reference to the Effects of Heat: Effects of Heat on Flour Proteins. *Cereal Chem.*, **8**, 1—23 (1931).

Hill, D. B., and Alsberg, C. L. A Modification of the Method of Preparing Gliadin. *J. Biol. Chem.*, **63**, lxvii (1925).

Hoffman, W. A., and Gortner, R. A. The Preparation and Analysis of the Various Proteins of Wheat Flour with Special Reference to the Globulin, Albumin and Proteose Fractions. *Cereal Chem.*, **4**, 221—9 (1927).

James, T. R., and Huber, L. X. A Study of the Physical Properties of Washed Gluten. *Cereal Chem.*, **4**, 449—60 (1927).

Johnson, A. H., and Bailey, C. H. Gluten of Flour and Gas Retention of Wheat-Flour Doughs. *Cereal Chem.*, **2**, 95—106 (1925).

Jong, H. L. B. de, and Klaar, W. L. Contribution to the Colloid Chemistry of Gluten. *Cereal Chem.*, **6**, 373—8 (1929); **7**, 222—44 (1920); **7**, 587—90 (1930).

Kent-Jones, D. W. The Strength of Flour. *Food Manfr.*, **1**, 101—2 (1927).

Kuhn, A., and Richter, G. The Colloid Chemical Properties of Wheat Gluten. *Kolloidchem. Beihefte*, **22**, 421—48 (1926).

Larmour, R. K. A Comparative Study of the Glutelins of the Cereal Grains. *J. Agric. Research*, **35**, 1091—1120 (1927).

Lüers, H. Colloid Chemistry of Bread : Colloid-Chemical Studies of Rye and Wheat Gliadin with Special Reference to Gluten and Baking Qualities. *Kolloid-Z.*, **25,** 177—96, 230—40 (1919) ; The Swelling of Gluten and its Importance in Relation to Baking Value. *Chem. Ztg.*, **44,** 324 (1920), and *Z. Elektrochem.*, **26,** 420—4 (1920).

Lüers, H., and Ostwald, Wo. The Colloid Chemistry of Bread : The Colloidal Swelling of Wheat Gluten. *Kolloid-Z.*, **27,** 34—7 (1920).

Mangels, C. E. Relation of Protein Content to Baking Quality of Flour from Hard Red Spring and Durum Wheats. *Cereal Chem.*, **3,** 150—7 (1926) ; Relation of Water-Absorbing Capacity of Flour to Protein Content, Baking Quality and Loaf Weight. *Ibid.*, **5,** 75—7 (1928).

Modern, F., and Pauli, W. Electrodialysis of Gluten. *Anales asoc. quim. Argentina*, **23,** 93—148 (1925).

Olson, G. A. Wheat and Flour Investigations. Washington Agr. Expt. Sta., *Bull.*, **144,** 5—86 (1917).

Osborne, T. B., and Voorhees, C. G. The Proteids of Wheat Kernel. *J. Am. Chem. Soc.*, **15,** 392—471 (1893) ; **16,** 524—35 (1894).

Osborne, T. B. The Proteins of the Wheat Kernel. Carnegie Inst. Washington, *Publication* No. **84,** 1—119 (1907).

Remington, J. S. Some Factors Influencing the Strength of Wheat and Flour. *Ind. Chem.*, **2,** 35—8 (1926).

Sharp, P. F., and Gortner, R. A. Physico-Chemical Studies of " Strong " and " Weak " Flours : the Imbibitional Properties of the Glutens from " Strong " and " Weak " Flours. *J. Phys. Chem.*, **26,** 101—36 (1922) ; The Identity of the Gluten Protein Responsible for the Changes in Hydration Capacity Produced by Acids. *Ibid.*, **27,** 674—84 (1923) ; Effect of Yeast Fermentation on Imbibitional Properties of Gluten. *Cereal Chem.*, **1,** 29—37 (1924).

Snyder, F. A. Crude Gluten. *J. Am. Chem. Soc.*, **28,** 8—25 (1906).

Stockham, W. L. Some Factors Related to the Quality of Wheat and the Strength of Flour. N. Dakota Agr. Expt. Sta., *Bull.*, **139,** 1—69 (1920).

Sullivan, B., and Near, C. Chemical Constituents which Influence Gluten Quality. *Ind. Eng. Chem.*, **19,** 159—61 (1927).

Tague, E. L. The Solubility of Gliadin. *Cereal Chem.*, **2,** 117—27 (1925). The Gluten Quality of Flour and Its Isoelectric Point. *Ibid.*, **2,** 202—8 (1925) ; Isoelectric Points of Gliadin and Glutenin. *J. Am. Chem. Soc.*, **47,** 418—22 (1925).

Upson, F. W. The Colloidal Swelling of Wheat Gluten in Relation to Milling and Baking. Nebraska Agr. Expt. Sta., *Res. Bull.*, **8,** 3—26 (1916).

Upson, F. W., and Calvin, J. W. Colloidal Swelling of Wheat Gluten. *J. Am. Chem. Soc.*, **37,** 1295—1304 (1915).

Weaver, H. A., and Goldtrap, W. A. *J. Am. Assoc. Cereal Chem.*, **7,** 115—23.

Wood, T. B. The Chemistry of Strength of Wheat Flour. *J. Agr. Sci.*, **2,** 139—61, 267—77 (1907).

Wood, T. B., and Hardy, W. B. Physical State of Gluten. *Proc. Roy. Soc.*, **B. 81,** 38—43 (1909).

Woodman, H. E. The Chemistry of the Strength of Wheat Flour. *J. Agr. Sci.*, **12,** 231—43 (1922).

STALING OF BREAD

Barnard, H. E., and Bishop, H. E. The Effect of Bread Wrapping on the Chemical Composition of the Loaf. *Ind. Eng. Chem.*, **6,** 736—46 (1914).

Bryant, J. P. Starch and Staleness. *The Northwestern Miller*, **147,** 747 (1926).

Hanausek, T. F. Microscopy of Starch in Bread. *Arch. Chem. Mikros.*, **8,** 72—8 (1915).

Karacsonyi, L. The *Rôle* of Carbohydrates and Proteins in the Staling of Bread. *Z. Untersuch, Lebensm.*, **56,** 479—84 (1928); Staling and Hydrogen-Ion Concentration. *Cereal Chem.*, **5,** 477—81 (1928); Changes of Acidity During Storing in Bread. *Magyar Chem. Folyoirat.*, **35,** 22—5 (1919).

Katz, J. R. The Cause of the Staling of the Crust of Bread and the Possibility of Preventing this Change. *Z. Elektrochem.*, **19,** 663 (1913); The Velocity of Staling of Bread. *Verslag. Akad. Wetenschappen. Amsterdam*, **23,** 652—5 (1914); The Staling of Bread is Connected with a Change which takes Place not only with Wheat and Rye Starch but also with All Varieties of Starch, but it Leads to practically Important Results only in the case of Wheat and Rye Starch. *Ibid.*, **23,** 655—8 (1914); The Staling of Bread as a Physiologico-Chemical Problem. *Z. physiol. Chem.*, **95,** 104—29; 136—46, 147—51 (1915); Has Light an Effect upon the Staling of Bread? *Ibid.*, **96,** 288—91 (1916); Remarkable Property of Aldehydes to Prevent the Staling of Bread. *Ibid.*, **96,** 314—22 (1916); Gelatinisation and Retrogradation of Starch in Relation to the Problem of Bread Staling. Walton's "A Comprehensive Survey of Starch Chemistry," Vol. I., 100—117 (New York, 1928); Physical Chemistry of Starch and of Bread-Baking: (1) Alteration of X-ray Spectrum of Starch in the Baking and Staling of Bread. *Z. physik. Chem.*, **150,** 37—59 (1930); (2) The Modification of Starch with V-Spectrum (Gelatinisation Spectrum) is the Equilibrium Form at a High, and that with B-Spectrum (Retrogradation Spectrum) at a Low Temperature. *Ibid.*, **150,** 60—66 (1930); (3) First and Second Stages of Gelatinisation. *Ibid.*, **150,** 67—80 (1930).

Lorenz, R. The Physical Chemistry of Bread. *Chem. Ztg.*, **37,** 783 (1913).

Lüers, H., and Ostwald, Wo. The Colloid Chemistry of Bread: Meals of Poor Baking Quality. *Kolloid-Z.*, **26,** 66—7; **27,** 34—7 (1920).

Neumann, M. P. Changes in Bread on Ageing. *Z. ges. Getreidew.*, **6,** 119—22 (1914).

Ostwald, Wo. Colloid Problems in the Preparation of Bread. *Kolloid-Z.*, **25,** 26—45 (1919).

Peper, J. P. Determination of the Agr. of Bread. *Chem. Weekblad.*, **23**, 163—8 (1926).

Platt, W. Staling of Bread. *Cereal Chem.*, **7**, 1—34 (1930).

Schweizer, C. The Staling of Bread and Its Prevention. *Mitt. Lebensm. Hyg.*, **21**, 1—27 (1930).

Verschaffelt, E., and Teutem, E. V. The Change in the Microscopic Structure of Bread as it becomes Stale. *Z. physiol. Chem.*, **95**, 130—5 (1915).

Ziegelmayer, W. The action of Plant Lecithin on the Swelling and Surface Tension of Glutin and the Starches of Flour : the Investigation of Agglutinants in Cooking. *Kolloid-Z.*, **53**, 224—31 (1930).

EMULSIONS

Astruc, Aubouy, and Dunnan. Spontaneous Cream Separation. *Ann. fals.*, **7**, 418—20 (1914).

Behre, A. *et al.* The Composition of the Mayonnaise of Meat Salad. *Z. Untersuch. Lebensm.*, **44**, 333—6 (1922).

Bennion, E. B. Function of Oils and Fats and Emulsions of Oil and Water in Bread Making, with Special Reference to Gluten Formation and Modification. *J. Soc. Chem. Ind.*, **45**, 435—8 T (1926) ; Emulsification in the Confectionery and Baking Industries. *Food Manfr.*, **4**, 247—9 (1929).

Bitting, A. W. Mayonnaise as Emulsion. *The Canner*, **67** (15), 32—3 (1928).

Bohrisch, P. Stability of Codliver Oil-Phosphorus Emulsion. *Pharm. Ztg.*, **73**, 778—9 (1928).

Branch, L. L. Production of Commercial Mayonnaise. *Am. Food J.*, **19**, 460—1 (1924).

Burton, L. V. Mechanical Handling of Mayonnaise Dressing. *Food Inds.*, **1**, 60—1 (1928).

Cobb, R. M., and Hunt, F. S. The Dispersion of Powdered Egg-Yolk. *J. Am. Leather Chem. Assocn.*, **21**, 18—22 (1926).

Davis, C. E. Shortening—its Definition and Measurement. *Ind. Eng. Chem.*, **13**, 797—9 (1921).

Davis, C. E., and Mavesty, D. J. Leavens : Their Action and Measurement. *Ibid.*, **14**, 210—2 (1922).

Dixon, H. H., and Bennett-Clark, T. A. Electrical Properties of Oil-Water Emulsions with Special Reference to the Structure of the Plasmatic Membrane. *Sci. Proc. Roy. Dublin Soc.*, **19**, 421—40 (1930).

Dyk, J. C. V. A Study of the Function of Emulsions of Oil and Water in Breadmaking, with Special Reference to Gluten Formation and Modification. *J. Soc. Chem. Ind.*, **49**, 421—2 T (1930).

Edin, H. Further Investigation on Fat Emulsions of the " Itamin " Type Used for Feeding Calves. *Kgl. Landtbruks.-Akad. Handl. Tids.*, **68**, 763—93 (1929).

Eichstädt, A. Emulsions : (1) Conditions for Formation of Various Types of Emulsion from Fat and Skim Milk and the Effect of Emulsifying Media on Milk-in-Fat Emulsions. *Milchwirtschaft. Forsch.*, **9**, 388—95 (1930).

Fejer, A. V. Influence of the Melting-Point of Non-Emulsified Fats on their Rate of Disappearance from the Stomach. *Biochem. Z.*, **53**, 168—78 (1914).

Filinski, W., and Markert, W. Gastric Digestion under the Influence of Fats. *Compt. rend. soc. biol.*, **96**, 344—6 (1927).

Freudenberg, E. Physiology of Digestion in Infants : (1) Digestion of Fats. *Z. Kinderheilk.*, **43**, 437—56 (1927).

Fürth, O., and Scholl, R. The Influence of Bile Acid Salts on the Diffusion and Resorption Processes. A Contribution to the Physiology of Fat Digestion. *Biochem. Z.*, **222**, 430—56 (1930).

Gage, S. H., and Fish, P. A. Fat Digestion, Absorption and Assimilation in Man and Animals as Determined in the Dark-Field Microscope and a Fat-Soluble Dye. *Am. J. Anatomy*, **34**, 1—85 (1924).

Graff, M. B. Chemistry and the Shortening Industry. *Ind. Eng. Chem.*, **20**, 1309—12 (1928).

Gray, D. M. Chemical Aspects of Mayonnaise. *Oil Fat Ind.*, **4**, 410—25 (1927).

Gray, D. M., and Southwick, C. A. Consistency and Stability of the Mayonnaise Emulsion. *Proc. 3rd Ann. Convention Mayonn. Prod. Manuf. Assocn.*, (**1928**), 33—47 ; Scientific Research Affords Means of Measuring Stability of Mayonnaise. *Food Inds.*, **1**, 300—4 (1929) ; The Importance of Control in Mayonnaise Manufacture. *Glass Packer*, **2**, 311—4 (1929).

Grindrod, G. Theory of Fat Separation in Emulsions. *Canning Age* (October, 1927), p. 822.

Guthrie, E. S. Air in Butter. *J. Dairy Sci.*, **13**, 461—70 (1930).

Hattori, K., and Kani, J. Theory of Emulsions. *J. Pharm. Soc. Japan*, No. **546**, 667—71 (1927).

Haupt, H. Use of Plant Lecithin in Confectionery. *Deut. Nahrungsmittel-Rundsch.* (**1930**), 17—20.

Hazeloop, B., and Püper, M. Some Comments on the Emulsion Chapter of the Dutch Pharmacopeia. *Pharm. Weekblad.*, **65**, 217—22 (1928).

Kernot, J. C., and Knaggs, J. Gelatins of Different Origin as Emulsifying Agents. *J. Soc. Chem. Ind.*, **47**, 96—100 T (1928).

King, N. Colloid Chemistry of Butter. *Kolloid-Z.*, **52**, 319—32 (1930).

Knight, W. A. Emulsio petrolei, Brit. Pharm. Codex. *Pharm. J.*, **121**, 297 (1928).

Krajewsky, N. A., and Wvedensky, N. Surface Tension of Bile Acids. *Biochem. Z.*, **191**, 241—9 (1927).

Krantz, J. C., and Gordon, N. E. A Study of the Emulsifying Properties of Certain Salts of Arabic Acid. *J. Am. Pharm. Assocn.*, **18**, 463—8 (1929).

Krantz, J. C. Effect of Buffering the Outer Phase on the Stability of Certain Emulsions. *Ibid.*, **19**, 1181—5 (1930).

Landstrom, A. W. Homogeniser as Improvement. *Baking Tech.*, **1**, 350—7 (1922) ; Homogeniser Reduces Losses. *Ibid.*, **2**, 22—4 (1923) ; Homogenisation Applied to Baking. *J. Am. Assocn. Cereal Chem.*, **8**, 128—34 (1923).

Lepper, H. A. Salad Dressings and Their Analysis. *J. Assocn. Official Agr. Chem.*, **5**, 248—53 (1921).

Lloyd, J. W., Ostwald, W., and Haller, W. Physics in Pharmacy. *J. Am. Pharm. Assocn.*, **18**, 862—75 (1929).

Locatelli, P. The Metabolism of Fats. *Arch. sci. biol. Italy*, **12**, 407—11 (1928).

Ludlum, S. D. W., Taft, A. E., and Nugent, R. L. Human Blood Serum as a Colloidal System. *Am. Coll. Symp. Monog.*, **7**, 233—48 (1929) ; The Chylomicron Emulsion. *J. Phys. Chem.*, **35**, 269—88 (1931).

Manhart, V. C. Variability in Composition of Butter from the Same Churning in Relation to Working. *J. Dairy Sci.*, **11**, 52—61 (1928).

Mark, K. L. Emulsification in Mayonnaise. *J. Home Econ.*, **13**, 447 (1921).

Nomura, T. Experimental Studies of Intravenous Injection of Fat with Special Reference to Parenteral Feeding : (1) Variation in the Fat Content of the Blood after Fat Injection. *Tohoku J. Exptl. Med.*, **12**, 247—53 (1929).

Pech, J. L. Extraction from Oleaginous Fruits of Substances which Permit the Emulsification of Certain Oils in Water. *Compt. rend. soc. biol.*, **89**, 983—4 (1923).

Pick, L. A Method for Measuring the Absorption Ability of Fats for Water. *Allgem. Öl. Fett.-Ztg.*, **26**, 577—9 (1929).

Platt, W. Biscuit and Cake Manufacture. *Chem. Age (N.Y.)*, **30**, 157—60, 203—6 (1922).

Mellanby, J. Petroleum Emulsion in the Small Intestine. *J. Physiol.*, **64**, (3), xxxiii—xxxiv (1927).

Mohr, W., and Eichstädt, A. Emulsions : (2) Stability of Emulsions in Relation to Viscosity of Fat, Surface Tension and the Formation of Adsorption Films. *Milchwirtschaft. Forsch.*, **9**, 396—408 (1930).

Obst, W. The Effect of Codliver Oil Emulsion in Raising and Fattening Stock. *Fortschritte Landw.*, **2**, 432—4 (1927).

Pease Laboratories. Practical Methods of Mayonnaise Manufacture. *Glass Container*, **9** (6), 12, 18, 20—2 ; (7), 18—24 (1930).

Platt, W. Shortening in Sweet Doughs. *Baking Tech.*, **2**, 216—9 (1923).

Platt, W., and Fleming, R. S. The Action of Shortening in the Light of the Newer Theories of Surface Phenomena. *Ind. Eng. Chem.*, **15**, 390—4, 1089 (1923).

Price, H. I., and Lewis, W. C. McC. The Physico-Chemical Behaviour of Lecithin : (1) The Capillary Activity of Lecithin as a Function of pH. *Biochem. J.*, **23**, 1030—43 (1929).

Robinson, S. K. Practice in Mayonnaise Manufacture. *Am. Food J.*, **19**, 185—7 (1924).

Roth, G. B. Petrolatum Emulsion on Surviving Intestine. *J. Pharmacol.*, **33**, 277 (1928).

Ruehe, H. A., and Stiritz, B. A. Some Studies in Reducing the Stability of Butterfat Emulsions. *J. Dairy Sci.*, **8**, 330—43 (1925).

Schlagintweit, E. Effect of Paraffin Oil on the Intestine. *Arch. exptl. Path. Pharmakol.*, **124**, 59—64 (1927).

Schulek, E., and Vastagh, G. Chemical Examination of Paraffin Oil Emulsions Prepared with Tragacanth. *Pharm. Zentralhalle*, **69**, 275—6 (1928).

Smith, C. Viscosity Factor in Emulsification. *Phil. Mag.* (7), **4**, 820—6 (1927).

Smith, E. L. A Factor Inhibiting the Emulsification of Cod-Liver Oil. *Quart. J. Pharm. Pharmacol.*, **3**, 373—4 (1930).

Smith, E. L., and Grinling, G. N. Studies in Emulsification: (1) Examination and Analysis of Pharmaceutical Emulsions. *Pharm. J.*, **125**, 91—2 (1930). *Quart. J. Pharm. Pharmacol.*, **3**, 345—61 (1930).

Smith, E. L., and Hazley, V. Some Observations on the Manufacture of Pharmaceutical Emulsions. *Ibid.*, **3**, 362—72 (1930).

Spranger, W. Body Fats. Physiology of Fat Deposition. *Biochem. Z.*, **208**, 164—78 (1929).

Turner, R. H. The Action of Bacteria on Fat. A Microscopic Study of Emulsion of Oil in an Agar Medium. *J. Infec. Diseases*, **44**, (2), 134—41 (1929).

Verzár, F., and Kuthy, A. The *Rôle* of Bile Salts in Fat Absorption. *Biochem. Z.*, **205**, 369—79 (1929).

Waggoner, C. S., and Glover, C. C. Analysis of Emulsions of Codliver Oil and Malt Extract. *J. Am. Pharm. Assocn.*, **15**, 754—5 (1926).

Warren, L. E. Examination of Some Liquid Petrolatum-Agar Emulsions. *J. Am. Med. Assocn.*, **84**, 1682—3 (1925).

Weiner, P. Absorption and Deposition of Fat in the Intestine. *Z. Mikroskop. Anat. Forsch.*, **13**, 197—268 (1928).

Whitmore, W. F., and Linehan, R. E. Transparent Emulsions of Some Essential Oils. *Ind. Eng. Chem.*, **21**, 878—80 (1929).

Wilson, J. A. Egg Albumin and Egg Yolk as Emulsifying Agents in Fat-Liquoring. *J. Am. Leather Chem. Assocn.*, **22**, 559—66 (1927).

Zernik, F. Alleged Destruction of Vitamins in Codliver Oil Emulsion. *Pharm.-Ztg.*, **70**, 156 (1925).

Zhukov, I. I., and Bushmakin, I. N. Gelatin as an Emulsifying Agent. *J. Russ. Phys. Chem. Soc.*, **59**, 1061—9 (1927).

MILK

Baterman, G. M., and Sharp, P. F. A Study of the Apparent Viscosity of Milk as Influenced by Some Physical Factors. *J. Agr. Research*, **36**, 647—74 (1928).

Borrino, A. Researches on the Ultrafiltration of Milk. *Arch. ital. biol.*, **64**, 417—25 (1917).

Clark, W. M. "Synthetic Milk" as a Basis for Research. *J. Dairy Sci.*, **10**, 195—201 (1927).

Coste, J. H., and Shelbourn, E. T. Electrical Conductivity of Milk. *Analyst*, **44**, 158—64 (1919).

Evenson, O. L. Remade Milk. Intern. Assocn. Dairy Milk Insps. *Ann. Rept.*, **12**, 354—71 (1923).

Evenson, O. L., and Ferris, L. W. The Viscosity of Natural and Remade Milk. *J. Dairy Sci.*, **7**, 174—88 (1923).

Fricke, H., and Morse, S. An Experimental Study of the Electrical Conductivity of Disperse Systems : (1) Cream. *Phys. Rev.*, **25**, 361—7 (1925).

Gerber, V. The Significance of the Specific Electrical Conductance of Milk and a New, Practical Method for its Determination. *Z. Untersuch. Lebensm.*, **51**, 336—51 (1926) ; **54**, 257—70 (1927).

Grimmer, W., and Benduski, H. Physical Examination of Milk. *Milchwirtschaft. Forsch.*, **7**, 76—99 (1928).

Huyden, E. Study of the Variations in the Composition of Milk. *Ann. méd. vétérinaire*, **68**, 111—24, 163—9 (1923).

Jackson, L. C. *et al.* The Electrical Conductivity of Milk during its Concentration, with Suggestions for a Practical Method of Determining the End Point in the Manufacture of Sweetened Condensed Milk. *J. Soc. Chem. Ind.*, **33**, 59—60 (1914).

Kobler, B. Researches on the Viscosity and Surface Tension of Milk. *Arch. ges. Physiol. Pflüger's*, **125**, 1—72 (1908).

Krenn, J. Studies on the Electrical Conductance of Milk : (1) Detection of Milk Altered by Disease. *Z. Untersuch. Lebensm.*, **57**, 148—77 (1929) ; (2) Practical Measurements on the Milk of Cows from Different Herds. *Ibid.*, **59**, 32—62 (1930).

Mattick, E. C. V., and Hallett, H. S. Studies in Lactation : (1) Variations in Some Chemical and Physical Properties of the Milk from Individual Quarters of a Cow. *J. Dairy Research*, **1**, 35—49 (1930).

Mohr, W., and Oldenburg, F. Viscosity of Milk and Milk Products : (1) Skim Milk, Full-Cream Milk and Cream ; (2) Fresh and Treated Milk and Cream. *Milchwirtschaft. Forsch.*, **8**, 429—61, 576—92 (1929).

Mohr, W., and Brockmann, C. Cataphoresis of Cow Milk. *Ibid.*, **11**, 211—17 (1930).

Mork, R. Investigations on the Skimming of Milk with a Special View to the Testing of the Efficiency of Different Separators. *Meldinger Norges Landbruks* **7**, 1—60 (1927).

Nakayama, M. Physico-Chemical Studies of Cow Milk and its Preparations. *Ber. ges. Physiol. exptl. Pharmakol.*, **46**, 353 (1928).

Overman, O. R., Sanmann, F. P., and Wright, K. E. Studies on the Composition of Milk. Univ. Illinois Agr. Expt. Sta., *Bull.*, **325**, 1—174 (1929).

Palmer, L. S., and Eckles, C. H. Influence of the Stage of Gestation on the Composition and Properties of Milk. *J. Dairy Sci.*, **1**, 185—98 (1917).

Palmer, L. S. The Chemistry of Milk and Dairy Products from a Colloid Standpoint. *Ind. Eng. Chem.*, **16**, 631—5 (1924) ; Some Recent Advances in the Chemistry of Milk. *Ibid.*, **22**, 39—42 (1930).

Perov, S. S. Electrical Conductance of Milk and the External Medium. *Chem. Abs.*, **24**, 3571 (1930).

Porcher, C. "Le Lait au Point de Vue Colloidal." (Lyon, 1930). Pp. 530.

Richmond, H. D. Milk. Thorpe's "Dictionary of Applied Chemistry" (London, 1922), 362—81.

Rossi, A. Distribution of Electrolytes in Milk and in the Ultrafiltrate. *Boll. soc. ital. biol. sper.*, **5**, 475—9 (1930).

Schenk, A. Dispersoid-Chemical Methods for the Examination of Milk. *Milchwirtschaft. Forsch.*, **7**, 1—34 (1928).

Sharp, P. F. Physical Constants of the Milk as Influencing the Centrifugal Separation of Cream at Various Temperatures. *J. Dairy Sci.*, **11**, 259—64 (1928).

Sommer, H. H., and Royer, K. M. The Cream Plug : Its Causes and Prevention. *J. Dairy Sci.*, **10**, 416—30 (1927).

Strohecker, R. The Significance of the Electrical Conductance of Milk in Analysis. *Z. Untersuch. Lebensm.*, **49**, 342—52 (1925).

Taylor, H. B. Some Physico-Chemical Measurements on Milk. *J. Proc. Roy. Soc. N.S. Wales*, **46**, 174—92 (1914).

Wardlaw, H. S. H. The Diffusible Phosphorus of Cow Milk. *J. Proc. Roy. Soc. N.S. Wales*, **48**, 253—66 (1914) ; The Relation between the Fat Content and the Electrical Conductivity of Milk. *Proc. Linnean. Soc. N.S. Wales*, **43**, 613—25 (1918).

Whitaker, R., Sherman, J. M., and Sharp, P. F. Effect of Temperature on the Viscosity of Skim Milk. *J. Dairy Sci.*, **10**, 361—71 (1927).

ACIDITY RELATIONS IN MILK

Anderson, L. Coagulation of Milk by Acid. *Trans. Faraday Soc.*, **19**, 106—11 (1923).

Arnandi, C. Physico-Chemical Investigations on Lactic Fermentation. *Boll. 1st Sieroterap. Milanese*, **7**, 387—409 (1928).

Buchanan, J. H., and Peterson, E. E. The Buffers of Milk and Buffer Value. *J. Dairy Sci.*, **10**, 224—31 (1927).

Comel, M. The Buffer Action of Milk. *Bull. soc. ital. biol. sper.*, **3**, 908—11 (1928) ; The Buffer Action of Milk Plasma. *Ibid.*, **3**, 912—15 (1928) ; The Buffer Action of Milk Serum. *Ibid.*, **3**, 915—8 (1928) ; A Study of the Buffer Action of Milk. *Arch. ital. biol.*, **81**, 61—70 (1929).

Dam, W. V. The Acidity of Milk and Whey. *Verslag. Landw. Onderzoek. Rijklandbouwproefstat.*, **1918**, 1—24.

Duncombe, E. The Influence of Certain Factors on the Hydrogen Ion Concentration of Milk. *J. Dairy Sci.*, **7**, 86—93, 245—8 (1924).

Frei, W., and Lienhard, H. Studies on the Hydrogen Ion Concentration of the Calcium Chloride Serum from Normal and Pathological Cow Milk. *Biochem. Z.*, **178**, 1—27 (1926).

Matsumoto, B. The Hydrogen Ion Concentration and the Acid-Combining Capacity of Human Milk. *Biochem. Z.*, **177**, 118—31 (1926).

Rice, F. E., and Markley, A. L. The Relation of Natural Acidity in Milk to Composition and Physical Properties. *J. Dairy Sci.*, **7**, 468—83 (1924).

Sharp, P. F., and McInerney, T. J. The Relation of the Hydrogen Ion Concentration to the Titratable Acidity of Milk. *J. Biol. Chem.*, **75**, 177—84 (1927).

Schultz, E. W., and Chandler, L. R. The Acidity of Goat Milk in Terms of Hydrogen Ion Concentration with Comparison to that of Cow and Human Milk. *J. Biol. Chem.*, **46**, 129—31 (1921).

Sherwood, F. F., and Hammer, B. W. Citric Acid Content of Milk ; Iowa State Coll. Agr. Expt. Sta., *Res. Bull.*, **90**, 19—39 (1926).

Slyke, L. L. V., and Baker, J. C. Carbonic Acid and Carbonates in Cow Milk. *J. Biol. Chem.*, **40**, 335—44 (1919).

Sommer, H. H., and Ménos, J. The Effect of Dilution on the Titratable Acidity of Cows' Milk. *J. Dairy Sci.*, **14**, 136—55 (1931).

Supplee, G. C., and Bellis, B. The Citric Acid Content of Milk and Milk Products. *J. Biol. Chem.*, **48**, 453—61 (1921).

Szili, A. The Reaction of Human Milk. *Biochem. Z.*, **84**, 194—200 (1917).

Terry, R. W. Potential Acidity of Milk and a Standard Method for Its Determination. *J. Am. Pharm. Assocn.*, **8**, 538—47 (1919).

Tillmans, J., and Obermeier, W. The Hydrogen Ion Concentration of Milk. *Z. Untersuch. Lebensm.*, **40**, 23—34 (1920).

Vandevelde, J. J. Indicators of the Real and Total Acidity of Milk. *Lait*, **7**, 140—9 (1927).

Watson, P. D. Variations in the Buffer Value of Herd Milk. *J. Dairy Sci.*, **14**, 50—8 (1931).

Whittier, E. O., and Benton, A. G. Effect of Heating on the Hydrogen Ion Concentration and on the Titratable Acidity of Milk. *J. Dairy Sci.*, **9**, 481—8 (1926) ; The Formation of Acid in Milk by Heating. *Ibid.*, **10**, 126—38 (1927).

Whittier, E. O. Buffer Intensities of Milk and Milk Constituents : (1) Buffer Action of Caseinogen. *J. Biol. Chem.*, **83**, 79—88 (1929) ; The Solubility of Calcium Phosphate in Fresh Milk. *J. Dairy Sci.*, **12**, 405—9 (1929).

THERMAL STABILITY

Benton, A. G., and Albery, H. G. Studies on the Stability of Evaporated Milk during Sterilisation with Reference to the Hydrogen Ion Concentration, Alcohol Test and the Addition of Specific Buffers. *J. Biol. Chem.*, **68**, 251—63 (1926).

Burgwald, L. H. Some Factors which Influence the Feathering of Sweet Cream in Coffee. *J. Agr. Research*, **26**, 541—6 (1923).

Leighton, A., and Mudge, C. S. Heat Coagulation of Milk. *J. Biol. Chem.*, **56**, 53—73 (1923).

Magee, H. E., and Harvey, D. Studies on the Effect of Heat on Milk : (1) Some Physico-Chemical Changes Induced in Milk by Heat. *Biochem. J.*, **20**, 873—83 (1926).

Mattick, E. C. V., and Hallett, H. S. Effect of Heat on Milk : (*a*) On the Coagulability by Rennet ; (*b*) On the N, P, and Ca Content. *J. Agr. Sci.*, **19**, 452—62 (1929).

McInerney, T. J. Temperatures at which Milk of Different per Cents. of Acidity will Coagulate. *J. Dairy Sci.*, **3**, 220—6 (1920).

Orr., J. B., *et al.* The Effect of Pasteurisation on the Nutritive Value of Milk. *Scottish J. Agr.*, **9,** 377—85 (1926).

Palmer, L. S. A Study of Heat-Coagulable and Water-Soluble Protein of Cow Milk. Miss. Agr. Expt. Sta., *Bull.*, **151,** 37—8 (1917) ; The Effect of Heat on the Calcium Salts and Rennin Coagulability of Cow Milk. *Proc. Soc. Exptl. Biol. Med.*, **19,** 137—42 (1921).

Porcher, C. Action of Heat upon the Complex Calcium Caseinate + Calcium Phosphate. Greater Sensitivity of the Phosphate Micelles. *Compt. rend.*, **182,** 1247—9 (1926).

Rogers, L. A., Deysher, E. F., and Evans, F. R. The Relation of Acidity to the Coagulation Temperature of Evaporated Milk. *J. Dairy Sci.*, **4,** 294—309 (1921).

Sommer, H. H., and Hart, E. B. Effect of Heat on the Citric Acid Content of Milk. Isolation of Citric Acid from Milk. *J. Biol. Chem.*, **35,** 313—8 (1918). The Heat Coagulation of Milk. *Ibid.*, **40,** 137—51 (1919) ; *J. Dairy Sci.*, **5,** 525—43 (1923) ; Heat Coagulation of Evaporated Milk. Univ. Wisconsin Agr. Expt. Sta., *Res. Bull.*, **67,** 1—47 (1926).

Tapernoux, A., and Katrandjieff, K. Heat Coagulation of Milk as a Function of its Acidity. *Compt. rend. soc. biol.*, **101,** 828—9 (1929).

Tracy, P. H., and Ruehe, H. A. A Case of Cream Feathering. *Cream Milk Plant Monthly*, **17,** 21 (1928) ; Causes of Feathering of Coffee Cream. *Ibid.*, **19,** (9), 36—42 (1930) ; Factors Affecting the Heat Coagulation of Homogenised Coffee Cream. Illinois Agr. Expt. Sta., *Bull.*, **352,** 569—78 (1930).

Webb, B. H., and Holm, G. E. The Heat Stability and Feathering of Sweet Cream as Affected by Different Homogenisation Pressures and Different Temperatures of Forewarming. *J. Dairy Sci.*, **11,** 243—57 (1928).

Whitaker, R. The Feathering of Evaporated Milk in Hot Coffee. *J. Dairy Sci.*, **14,** 177—88 (1931).

Whittier, E. O., and Benton, A. G. The Rate of Acid Production in Heated Milk. *J. Dairy Sci.*, **10,** 343 (1927).

PASTEURISATION

Achard, G., and Stassano, H. Some Physico-Chemical Constants of Raw Milk and of Milk Pasteurised According to Different Methods. *Compt. rend. soc. biol.*, **92,** 708—10 (1925).

Beckett, D. A. Influence of Separation and Pasteurisation on the Size and Distribution of the Fat Globules in Milk and Cream. *Econ. Proc. Roy. Dublin Soc.*, **2,** 303—17 (1927).

Daniels, A. L., and Stearns, G. The Effect of Heat Treatment of Milk Feedings on the Mineral Metabolism of Infants. *J. Biol. Chem.*, **61,** 225—40 (1924).

Daum, K. The Effect of Heat upon Certain Constituents of Milk. *Abstracts of Theses, Univ. Chicago, Science Series*, **4,** 423—30 (1925/26).

Levine, V. E. Pasteurised Milk as a Food for Infants. *Arch. Pediatrics*, **44,** 273—81 (1927).

Matsuo, I. Biochemical Studies of Milk : The Effect of Heating upon the Constituents of Milk. *Osaka J. Med.*, **28**, 555—62 (1929).

Rahn, O., and Mohr, W. The Distribution of the Fat in Pasteurised Cream. *Milchwirtschaft. Forsch.*, **1**, 363—73 (1924).

Reed, W. H. E. The Effect of Different Pasteurising Temperatures on Several of the Physical Properties of Milk. Miss. Agr. Expt. Sta., *Res. Bull.*, **126**, 2—12 (1929).

Weinlig, A. F. Physical and Chemical Changes of Milk during Pasteurising. *Forsch. Geb. Milchw. Molkereiw.*, **2**, 127—69, 175—205 (1922).

Woll, F. W. The Effect of Pasteurisation and Sterilisation on the Viscosity and Fat Globules of Milk and Cream. Wisconsin Agr. Expt. Sta., *Ann. Repts.*, **12**, 164—73 (1895).

Yamamura, K. Effect of the Heating Temperature on Milk and Estimation of the Heating Temperature of Dairy Products. *J. Agr. Chem. Soc. Japan*, **6**, 462—73 (1930).

HOMOGENISED MILK

Baldwin, H. M. Some Observations on Homogenised Milk and Cream. *Am. J. Pub. Health*, **6**, 862—4 (1916).

Burr, A., and Weise, H. Studies on Homogenised Milk. *Molkerei Ztg.*, **28**, 367—8, 381—2 (1914).

Clayton, W. Homogenised Milk and Cream. *The Milk Industry* (Feb., 1923).

Doan, F. J. Viscolised Milk and its Detection. *J. Dairy Sci.*, **10**, 501—11 (1927) ; The Homogenising Process. *Pennsylvania Bull.*, **230**, 23—5 (1928) ; Some Factors Affecting the Fat Clumping in Milk and Cream Mixtures when Homogenised. *J. Dairy Sci.*, **12**, 211—30 (1929).

Sobbe, O. V. The Determination of the Degree of Homogenisation of Milk. *Milchwirtschaft. Zentr.*, **43**, 503—6 (1914).

Wiegner, G. The Change of Some Physical Properties of Cow Milk with Changes in the Degree of Dispersion of its Dispersed Phases. *Kolloid-Z.*, **15**, 105—23 (1914).

CASEIN (See also p. 465)

Berry, R. A. Casein and the Dairy Industry. *Scottish J. Agr.*, **9**, 167—71 (1926).

Bleyer, B., and Seidl, R. The Casein of Cow Milk. *Biochem. Z.*, **128**, 48—75 (1922) ; Ultramicroscopy of Casein. *Kolloid-Z.*, **30**, 117—8 (1922).

Bosworth, A. W., and Giblin, L. A. The Casein of Human Milk. *J. Biol. Chem.*, **35**, 115—7 (1918).

Dahlberg, A. D. The Manufacture of Casein from Butter-Milk or Skim Milk. U.S. Dept. Agr., *Bull.*, **661**, 1—32 (1918).

Dudley, H. W., and Woodman, H. E. The Specificity of Caseinogens. A Comparative Study of the Caseinogens of the Cow and the Sheep. *Biochem., J.*, **9**, 97—102 (1915).

Foreman, F. W. A New Method for Preparing Esters of Amino Acids. Composition of Caseinogen. *Biochem. J.*, **13**, 378—97 (1919).

Fouassier, M. Commercial Caseins. *Bull. soc. hyg. aliment.*, **16,** 218—29 (1928).

Haglund, E. Technique and Development of Commercial Casein. *Kgl. Landtbruks. Akad. Handl. Tids.*, **1913,** 457—512.

Høyberg, H. M. Casein Content of Danish Milk. *Z. Fleisch. Milchhyg.*, **35,** 381—3 (1925).

Leberman, F. The Precipitation of Casein at the Isoelectric Point. *Biochem. Z.*, **206,** 56—9 (1929).

Nabenhauer, F. P. Manufacture of Casein and Lactose from Skim Milk. *Ind. Eng. Chem.*, **22,** 54—6 (1930).

Porcher, C. Casein : Acid Casein and Rennet Casein and the Conversion of the Former into the Latter. *Chimie et industrie*, **19,** 589—98, 809—16 (1928).

Trendtel, F. The Individual Differences in the Isoelectric Point of Casein from Human Milk. *Biochem. Z.*, **180,** 371—6 (1927).

Zoller, H. F. Hydrogen Electrode Study of the Curdling in Casein Solutions at High Temperatures. *Science*, **52,** 614 (1920) ; Casein and the Dairy Industry. Bogue's " Theory and Application of Colloidal Behaviour " (New York, 1924), Vol. 2, 784—807.

RENNET COAGULATION

Alexander, J. The Rennin Coagulation of Milk. *Ind. Eng. Chem.*, **16,** 974 (1924). This was replied to by Palmer, *ibid.*, p. 974, Alexander replying, p. 1282.

Allemann, O., and Schmid, H. The Elasticity of the Coagulum produced in Milk by Rennet. *Landw. Jahrb. Schweiz.*, **30,** 357—83 (1916).

Baur, E., and Herzfeld, E. Coagulation of Milk by Rennet as a Process of Stimulus Transmission. *Z. physik. Chem.*, **98,** 460—73 (1921).

Clementi, A. Inhibitory Action of Bile on the Coagulation of Milk by Rennet. *Arch. fisiol.*, **23,** 475—87 (1925).

Dominicis, A. de, and Rotonda, C. La. The Phenomenon of Rennet Coagulation. *Ann. scuola agr. Portici.* (2), **17,** 1—20 (1922) ; Action of Heat, *Ann. chim. applicata*, **16,** 294—9 (1926).

Grimmer, W., and Kruger, M. The Action of Rennet. *Milchwirtschaft. Forsch.*, **2,** 457—81 (1925).

Grimmer, W., and Rudzik, W. The Action of Rennet. *Ibid.*, **3,** 361—403 (1926).

Grimmer, W., and Hinkelmann, E. The Action of Rennet. *Ibid.*, **6,** 274—301 (1928).

Guittonneau, G. The *Rôle* of Rennet and its Mode of Action in the Manufacture of Heated-Curd Cheeses. *Compt. rend.*, **180,** 1536—8 (1925).

Marui, S. Rennin : The Effect of Heating on the Coagulability of Casein. *Biochem. Z.*, **173,** 363—70 (1926) ; The Exchangeability of Calcium for Other Cations. *Ibid.*, **173,** 371—80 (1926) ; The Exchangeability of Phosphate and Other Substances. *Ibid.*, **173,** 381—8 (1926).

Mattick, E. C. V., and Hallett, H. S. Effect of Heat on Milk : On the Coagulability by Rennet. *J. Agr. Sci.*, **19,** 452—62 (1929).

Michaelis, L., and Mendelssohn, A. The Conditions for the Activity of the Rennin Enzyme. *Biochem. Z.*, **58,** 315—28 (1913—14).

Milroy, T. H. The Reaction and Calcium Content of Milk as Factors in the Coagulation Process. *Biochem. J.*, **9,** 215—28 (1915).

Moir, G. M. The Effect of Heat upon the Rennin Coagulation. *J. Dairy Research*, **2,** 68—75 (1931).

Mundinger, E. Action of Rennet. *Milchwirtschaft. Forsch.*, **4,** 369—90 (1927).

Palmer, L. S., and Richardson, G. A. The Colloid Chemistry of Rennet Coagulation. *Am. Coll. Symp. Monog.*, **3,** 112—34 (1925).

Porcher, C. " Le Lait au Point de Vue Colloidal. Recherches sur le Mechanisme de l'Action de la Presure." (Lyon, 1929). Pp. 530 ; The Synthetic Method in the Study of Milk. Milk from the Colloid Point of View. Researches on the Mechanism of the Action of Rennin. *Lait*, **10,** 47—68, 146—75, 291—323, 401—27 (1930).

Richardson, G. A., and Palmer, L. S. Rennin Action in Relation to Enterokinetic Phenomena. *J. Phys. Chem.*, **33,** 557—76 (1929).

Rideal, E. K., and Wolf, C. G. L. The Destruction of Rennin by Agitation ; A Case of Catalysis at an Air-Liquid Interface. *Proc. Roy. Soc.*, **A 106,** 97—116 (1924).

Ronna, P., and Gabbe, E. The Action of Calcium on the Coagulation of Milk by Rennet. *Biochem. Z.*, **134,** 39—75 (1922).

Rotonda, C. La. Rennin Coagulation : Influence of Hydrogen Ions on the Separation of Casein by the Action of Electrolytes. *Ann. chim. applicata*, **19,** 310—28 (1929).

Rüdiger, M., and Wurster, K. The Combining Powers of the Milk Calcium and Their Importance for Rennin Coagulation. *Biochem. Z.*, **216,** 367—99 (1929).

Schryver, S. B. Further Experiments on the Clotting of Caseinogen Solutions. *Biochem. J.*, **7,** 568—75 (1913) ; Some Investigations on the Phenomena of " Clot " Formation : (1) On the Clotting of Milk. *Proc. Roy. Soc.*, **B 86,** 460—81 (1913).

Sirks, H. A. The Determination of Rennet Activity. *Verslag. Land. Onderzoek. Rijkslandbouwproefsta*, No. **25,** 83—113 (1921).

Stella, G. A Colloidal Mixed Solution of Calcium Carbonate and Calcium Phosphate. *Kolloid-Z.*, **43,** 21—6 (1927).

Stone, J. B., and Alsberg, C. L. Observations of the Rennin Coagulation of Milk. Effect of Hirudin, of Heparin, of Cephalin and of Fat Removal. *J. Biol. Chem.*, **78,** 557—72 (1928).

Townsend, C. T. Action of Rennet Extract on the Paracasein of Skim Milk as Affected by Different Organic Acids. *Sci. Agr.*, **6,** 159—64 (1925).

Waele, H. de. The Coagulation of Milk. *Ann. physiol. physicochim. biol.*, **3,** 113—20 (1927).

Wahlin, J. G. The Effect of Rennin on Sodium Caseinate. *J. Bact.*, **16,** 375—86 (1928).

Washburn, R. M., and Bigelow, A. P. Studies of the Rennet Coagulation of Milk. Vt. Agr. Expt. Sta., *Bull.*, **170** (1912).

Wurster, K. Relation between the Calcium Content of Allgau Soils to the Calcium Content of Milk and its Coagulability by Rennin. *Milchwirtschaft, Forsch.*, **9**, 258—85 (1930).

Zaykovskii, I. Action of Rennin on the Proteins of Milk. *Fermentforschung*, **8**, 537—46 (1926).

RENNIN SUBSTITUTES

Akiba, B. The Rennet-Like Action of Pepsin. *Keio Ig., Tokyo*, **2**, 1387—91 (1922).

Barr, G. H. Loss of Fat in the Whey when Using Pepsin. *Agr. Gaz. Canada*, **4**, 660—2 (1917).

Bodansky, A. A Study of a Milk-Coagulating Enzyme of Solanum Elaeagnifolium. *J. Biol. Chem.*, **61**, 365—75 (1924).

Clifford, W. M. The Effect of Fluorides and Iodides on the Clotting of Milk by Pepsin. *Biochem. J.*, **22**, 1128—32 (1928).

Fascetti, G. Rennet Substitute. *Staz. sper. agr. ital.*, **49**, 13—6 (1916).

Fenger, F. Comparison between the Chemical and Physiological Characteristics of Pepsin and Rennin. *J. Am. Chem. Soc.*, **45**, 249—55 (1923).

Graber, H. T. The Rennetic Properties of Pepsin. *Ind. Eng. Chem.*, **9**, 1125—6 (1917).

Lucas, P. S. The Use of Pepsin as a Rennet Substitute in Cheddar Cheese Making. Oregon Agr. Expt. Sta., *Bull.*, **155**, 1—15 (1918).

Merker, H. M. Pepsin v. Rennet in Cheese Making. *J. Dairy Sci.*, **2**, 482—6 (1919).

Michaelis, L., and Rothstein, M. The Destruction of Rennin and Pepsin by Alkali. *Biochem. Z.*, **105**, 60—87 (1920).

CHEESE

Callister, C. P. The Manufacture of Cheddar Cheese. *Chem. Eng. Mining Rev.*, **20**, 317—9 (1928).

Camps. The Use of Calcium Chloride in Cheesemaking in Denmark. *Lait*, **4**, 895—7 (1924).

Geake, A. Enquiry into the Factors which Control the Texture of Cheddar Cheese. *J. Agr. Sci.*, **6**, 169—78 (1914).

Gow, J. P. Cheddar Cheese Making. Union S. Africa, Dept. Agr., *Bull.*, **11**, 1—16 (1927).

Knaysi, G., and Nelson, J. D. Increasing the Yield of Cheese by the Addition of Calcium Chloride to Milk. *J. Dairy Sci.*, **10**, 396—9 (1927).

Knudsen, S. Determination of the Hydrogen Ion Concentration of Cheese by the Quinhydrone Electrode. *Z. Untersuch. Lebensm.*, **50**, 300—6 (1925).

Koestler, A. G. Different Types of Milk, Their Relation to the Rennet, and Their Importance in Cheesemaking. *J. Dairy Sci.*, **8**, 28—36 (1925).

Marquardt, J. C. Soft Cheese Investigations. *J. Dairy Sci.*, **10**, 309—30 (1927).

Platon, B. Experiments on Cheese Ripening with Milk containing added Calcium Chloride. *Medd. Centralanstalt. försöksväsendet jordbruks*, No. **32**, 1—16 (1927).

Price, W. V. Concerning the Addition of Calcium Chloride to Milk for Cheese Making. *J. Dairy Sci.*, **10**, 373—6 (1927).

Slyke, L. L. V. The Chemistry of Sour Milk. N.Y. Agr. Expt. Sta., *Tech. Bull.*, **140**, 1—14 (1928).

Sommer, H. H. A case of Sandiness in Processed Cheese. *J. Dairy Sci.*, **13**, 288—90 (1930).

Steinegger, R. The Formation of Holes in Emmenthaler Cheese. *Milchw. Zentr.*, **43**, 57—9 (1914).

Templeton, H. L., and Sommer, H. H. Some Observations on Processed Cheese. *J. Dairy Sci.*, **13**, 203—20 (1930).

Watson, P. D. Use of Quinhydrone Electrode for Following Changes of pH in Swiss Cheese. *Ind. Eng. Chem.*, **19**, 1272—4 (1927).

Watson, P. D., and Leighton, A. Some Observations on the Freezing Points of Various Cheeses. *J. Dairy Sci.*, **10**, 331—4 (1927).

Watson, P. D. The Relation of the Hydrogen Ion Concentration to the Texture of Emmenthal or Swiss Cheese. *Ibid.*, **12**, 289—303 (1929).

Yegunov, M. The Movement of Substances Through Cheese : The Theory of Salting-Down Cheese. *Visti Odeskii Silsko-Gospodar' skii Inst.*, **1**, 90—3 (1925—26).

WHEY AND WHEY PROTEINS

Bell, R. W., and Peter, P. N. Nature of the Neutralisation Precipitate and its Effect on the Recovery of Milk-Sugar from Grain-Curd-Casein Whey. *Ind. Eng. Chem.*, **20**, 510—2 (1928).

Benning, M. Properties of Whey and their Technical Importance in Manufacturing Cheese. *Landw. Jahrb. Bayern.*, **16**, 31—55 (1926).

Berry, R. A. The Production, Composition and Utilisation of Whey. *J. Agr. Sci.*, **13**, 193—239 (1923).

Bleyer, B., and Kallmann, O. Some Little Known Constituents of Milk. *Biochem. Z.*, **155**, 54—79 (1925).

Grimmer, W., Kurtenacker, C., and Berg. R. Serum Proteins of Milk. *Biochem. Z.*, **137**, 465—83 (1923).

Grimmer, W., and Hecht, A. The Protein Substances in Whey *Milchwirtschaft. Forsch.*, **4**, 564—82 (1927).

Kaku, T. Whey Albumose. *J. Pharm. Soc. Japan*, No. **504**, 93—106 (1926).

Lindet, L. The Solubility of the Proteins of Milk in the Constituents of the Serum : The Decrease in their Solubility under the Influence of Calcium Chloride. *Bull. soc. chim.*, **13**, 929—35 (1913) ; The Albuminoids of Milk, *Lait*, **1**, 161—70 (1921).

Mitamura, K. Influence of Neutral Salts upon the Alcohol Coagulation of Fresh Milk. *Sapporo J. Forestry Agr.*, **19**, 1—36 (1927).

Okuda, Y., and Zoller, H. F. The Relation of Hydrogen Ion Concentration to the Heat Coagulation of Proteins in Swiss Cheese Whey. *Ind. Eng. Chem.*, **13**, 515—9 (1921).

Rossi, P. Whey of the Cheese Factory and Some By-Products of the Dairy Industry : Their Properties and Nutritional Value. *Lait*, **10**, 136—42, 278—88 (1930).

COLLOID PROBLEMS OF CREAM RISING AND CHURNING

Babcock, C. J. The Whipping Quality of Cream. U.S. Dept. Agr., *Bull.*, **1075** (1922).

Bartsch, O. Foam. *Kolloidchem. Beihefte*, **20**, 1—49 (1924).

Behrendt, H. Surface Tension of Milk. *Z. Kinderheilk*, **33**, 209—17 (1922).

Boysen, H. The Distribution of Water in Butter. *Milchwirtschaft. Forsch.*, **4**, 221—48 (1927).

Brouwer, E. The Nature of the Agglutination of Fat Globules : The Relation of the Serum Globulin to the Creaming of Milk. *Chem. Abs.*, **20**, 2709 (1926) ; Comparative Studies of the Influence of Dipped and of Centrifuged Skim Milk on the Creaming of Washed Milk-Fat Globules. *Ibid.*, 2709.

Burg, B. van der. Milk as an Emulsion. The Phenomenon of Cream Rising. *Lait*, **7**, 452—66 (1927).

Burri, R., and Nussbaumer, T. The Traube Stalagmometer Investigation of Surface Tension and Viscosity of Milk. *Biochem. Z.*, **22**, 90—102 (1909).

Burri, R. Effect of Temperature on the Creaming of Milk. *Milchwirtschaft. Zentr.*, **45**, 33—9 (1916).

Dahlberg, A. C., and Hening, J. C. Viscosity, Surface Tension and Whipping Properties of Milk and Cream. N.Y. Agr. Expt. Sta., *Tech. Bull.*, **113**, 1—42 (1925).

Dam, W. van, and Sirks, H. A. The Friesish System of Cream Separation. *Verslag. Land. Onderzoek. Rijkslandbouwproefsta*, **26**, 106—86 (1922).

Dumaresq, F. K. M. The Viscosity of Cream. *Proc. Roy. Soc. Victoria (New Series)*, **25**, 307—22 (1913).

Eichstädt, A. See pp. 491 and 493.

Friese, W. The Composition of Milk Membranes. *Milchwirtschaft. Forsch.*, **1**, 316—29 (1924).

Gortner, R. A. The Application of Colloid Chemistry to Some Agricultural Problems. *Am. Coll. Symp. Monog.*, **1**, 392—412 (1923).

Guthrie, E. S., and Sharp, P. F. The Effect of the Hydrogen Ion Concentration on the Churning Time of Cream. *J. Dairy Sci.*, **14**, 1—13 (1931).

Hattori, K. Membranes of the Fat Globules of Milk. *J. Pharm. Soc. Japan*, No. **516**, 123—70 (1925) ; Supplementary Experiment on the Envelope Substance of Milk-Fat Globules. *Ibid.*, **49**, 332—6 (1929).

Hattori, K., and Ogimura, Y. Hull Substance of Milk-Fat Globules. *Ibid.*, **49**, 1147—9 (1929).

Hekma, E., and Brouwer, E. The Envelopes of Foam Bubbles in Milk. *Verslag.Land. Onderzoek. Rijkslandbouwproefsta*, No. **18**, 46—59 (1923).

Hekma, E. Agglutination of Fat Globules of Milk by Serum. *Arch. néerland. physiol.*, **10**, 444—5 (1925).

Hekma, E., and Sirks, H. A. Quantitative Investigation on the Influence of Blood Serum and Heated Blood Serum upon the Rising of Cream. *Ibid.*, **29**, 137—52 (1924).

Hittcher, K. Exhaustive Studies on the Buttermaking Process. *Landw. Jahrb.*, **51**, 489—562 (1918).

Jensen, O. The Theory of Cream Rising and Removal. *Milchwirtschaft. Forsch.*, **41**, 712—9 (1912).

Jorgensen, G. Experiments for Greater Churning Yields. *Molkerei-Ztg.*, **40**, 1772—3 (1926).

King, N. The Structure of Butter. *Milchwirtschaft. Forsch.*, **8**, 423—8 (1929) ; Concerning the Fat Globules in Butter. *Ibid.*, **8**, 95—8 (1929).

Kobler, B. The Viscosity and Surface Tension of Milk. *Arch. ges. Physiol. Pflüger's*, **125**, 1—72 (1908).

Leete, C. S. Foaming of Milk and Cream. U.S. Dept. Agr., *Circ.*, **108**, 1—6 (1930).

Mattick, A. T. R. Apparent Ropiness (Thread Formation) in Milk due to Surface Influence. *J. Agr. Sci.*, **16**, 459—65 (1926).

Mohr, W. Whipped Cream. *Molkerei-Ztg.* (*Hildesheim*), **39**, 259—62 (1925).

Mohr, W., and Brockmann, C. Surface Tension Measurements in Milk. *Milchwirtschaft. Forsch.*, **10**, 72—95 (1930) ; Contributions to the Phenomenon of Churning. *Ibid.*, **10**, 173—9 (1930) ; Contribution to the Problem of Foam. *Ibid.*, **11**, 48—61 (1930).

Mohr, W., and Eichstädt, A. The Stability of Emulsions in Relation to the Viscosity of the Fat, the Surface Tension and the Formation of Adsorption Films. *Ibid.*, **9**, 396—408 (1930).

Palmer, L. S. The Chemistry of Churning. Miss. Agr. Expt. Sta., *Bull.*, **163**, 40—1 (1919).

Palmer, L. S., and Anderson, E. O. Factors Influencing the Viscosity of Milk and their Relation to Creaming. Minnesota Agr. Expt. Sta., *Ann. Rept.*, **1922**, 1—39. Physico-Chemical Factors Influencing Cream Rising : (1) Viscosity. *J. Dairy Sci.*, **9**, 1—14 (1926) ; with **Hening, J. C.** (2) Relation of Plasma Colloids to Pasteurisation Effects. *Ibid.*, **9**, 171—91 (1926).

Porcher, C. The Formation of Fatty Material in Milk. *Lait*, **5**, 1—30 (1925).

Pratolongo, U. Studies on Butter Formation. Preliminary Note. *Giorn. chim. ind. applicata*, **6**, 3—10 (1924).

Pyne, G. T., and Lyons, J. Studies in Cream Viscosity. *J. Dept. Lands Agr. Ireland*, **27**, 121—8 (1928).

Rahn, O. Cream Formation. *Forsch. Geb. Milchw. Molkereiwes*, **1**, 133—54 (1921), 213—33 (1921) ; *Kolloid-Z.*, **30**, 110—4 (1922) ; The Significance of Surface Tension Phenomena in Dairy Work. *Kolloid-Z.*, **30**, 341—6 (1922) ; Process of Churning Butter : (1) A

Surface Tension Theory. *Forsch. Geb. Milchw. Molkereiwes*, **1**, 309—25 (1921); (2) Effect of Temperature. *Ibid.*, **2**, 76—94 (1922); The Division of Fat in Milk. *Milchwirtschaft. Forsch.*, **2**, 383—404 (1925).

Sakurei, K. The Rate of Separation of Emulsions such as Milk, Blood and Milky Plant Juices. *Biochem. Z.*, **149**, 525—33 (1924).

Sanmann, F. P., and Ruehe, H. A. Some Factors Influencing the Foam on Milk. *J. Dairy Sci.*, **13**, 48—63 (1930).

Schmid, F. The Microscopical Investigation of Cow Milk. *Schweiz. Milch. Ztg.*, **50** (17), 18 (1924).

Shaw, R. H., and Eckles, C. H. A Chemical and Physical Study of the Large and Small Fat Globules in Cow Milk. U.S. Dept. Agr., *Bull.*, **111**, 5—16 (1909).

Sirks, H. A. The Agglutination of the Fat Globules and Their Electric Charge. *Verslag. land. onderzoek. Rijkslandbouwproefsta.*, **29**, 137—52 (1924).

Soxhlet, F. Investigations on the Nature of the Globules in Milk and a New Theory of Churning. *Landw. Vers.*, **19**, 118—55 (1876).

Storch, V. Investigations on Some Frequent Viscosity Errors and their Cause; also the Structure of Milk Globules. *Milch.-Ztg.*, **26**, 273 (1897).

Titus, R. W., Sommer, H. H., and Hart, E. B. The Nature of the Protein Surrounding the Fat Globules in Milk. *J. Biol. Chem.*, **76**, 237—50 (1928).

Troy, H. C., and Sharp, P. F. Physical Factors Influencing the Formation and Fat Content of Gravity Cream. *J. Dairy Sci.*, **11**, 189—229 (1928).

Vvdenskii, I. N. The Surface Energy of Some Physiological Fluids. *Biochem. Z.*, **188**, 270—8 (1927).

Weigman, H. H. The Degree of Dispersion of Fat in Milk. *Milchwirtschaft. Forsch.*, **4**, 259—311 (1927).

ICE CREAM

Bateman, G. M., and Sharp, P. F. Some Observations on the Consistency of Cream and Ice Cream Mixtures. *J. Dairy Sci.*, **11**, 380—96 (1928).

Davel, H. B. The Manufacture of Ice Cream. Union S. Africa Dept. Agr., *Bull.*, **53**, 7—30 (1929).

Given, M. H. Textures of Ice Creams as Influenced by Some Constituents. *Ind. Eng. Chem.*, **20**, 966—8 (1928).

Hening, J. C. Effect of Homogenising Ice Cream Mixes Before and After the Addition of Gelatin or Sugar and Before and After Condensing. *J. Dairy Sci.*, **11**, 299—312 (1928).

Hening, J. C., and Dahlberg, A. C. The Effects of Certain Salts on the Physical Properties of Ice Cream Mixes. *Ibid.*, **12**, 129—39 (1929).

Hening, J. C. Some Observations on the Basic Viscosity of Ice Cream Mixes. *Ibid.*, **14**, 84—92 (1931).

Holdaway, C. W., and Reynolds, R. R. Effect of Binders upon the Melting and Hardness of Ice Cream. Virginia Agr. Expt. Sta., *Bull.*, **211**, 3—19 (1916).

Leighton, A., and Peter, P. N. Factors Influencing the Crystallisation of Lactose. *Proc. World's Dairy Cong.*, **1**, 477—85 (1923).

Leighton, A., and Williams, O. E. The Effect of Temperature on the Basic Viscosity of Ice Cream Mixes. *J. Phys. Chem.*, **31**, 1663—8 (1927).

Leighton, A. Application of Physical Chemistry to Ice Cream. *Ind. Eng. Chem.*, **22**, 48—51 (1930).

Martin, W. H., and Caulfield, W. J. The Use of Egg Yolk in Ice Cream. *J. Dairy Sci.*, **12**, 193—201 (1929).

Moore, H. C., Combs, W. B., and Dahle, C. D. Relation between the Gold Number of Gelatin and its Value in the Ice Cream Mix. *J. Dairy Sci.*, **8**, 500—11 (1925).

Mueller, W. S., and Button, F. C. The Use of Dehydrated Egg Products in the Manufacture of Ice Cream. *J. Dairy Sci.*, **12**, 320—335 (1929).

Parisi, P. Influence of the Reaction on the Technique of the Making of Lactose, Condensed Milk and Ice Cream. *Giorn. chim. ind. applicata*, **12**, 225—35 (1930).

Palmer, L. S., and Dahle, C. D. Factors Influencing Lactose Crystallisation in the Occurrence of Sandy Ice Cream. Minnesota Agr. Expt. Sta., *Ann. Rept.*, **1922**, 1—41.

Peter, P. N. Solubility Relationships of Lactose-Sucrose Solutions. (1) Lactose-Sucrose Solubilities at Low Temperatures. *J. Phys. Chem.*, **32**, 1856—64 (1928).

Reid, R. G. The Manufacture of Ice Cream. *Proc. 4th Intern. Cong. Refrig.* (London, 1924), **1**, 928—44.

Reid, W. E., and Mcseley, W. K. The Effect of Processing on the Dispersion of Fat in an Ice Cream Mix. *Ibid., Res. Bull.*, **91**, 1—25 (1926).

Reid, W. H. E., and Scism, S. F. The Effect on the Viscosity, Bacterial Flora and Quality of the Resulting Ice Cream when the Ice Cream Mixture is Re-Emulsified, Re-Viscolised or Re-Homogenised. Miss. Agr. Expt. Sta., *Res. Bull.*, **82**, 1—22 (1925).

Reid, W. H. E. What Causes Sandy Ice Cream. *Ice Cream Trade J.*, **26**, 60—4 (1930).

Reid, W. H. E., and Garrison, E. R. Effect of Processing Ice Cream Mixtures at Different Pressures when the Milk-Solids-Not-Fat Content is Varied. *Ibid., Res. Bull.*, **128**, 1—44 (1929).

Sherwood, F. F., and Smallfield, H. L. Factors Influencing the Viscosity of Cream and Ice Cream. *J. Dairy Sci.*, **9**, 68—77 (1926).

Sommer, H. H., and Young D. M. Effect of Milk Salts on the Whipping Ability of Ice Cream Mixes. *Ind. Eng. Chem.*, **18**, 865—6 (1926).

Troy, H. C., and Sharp, P. F. α- and β-Lactose in Milk Products. *J. Dairy Sci.*, **13**, 140—57 (1930).

Turnbow, G. D., and Nielson, K. W. Viscosity and the Ice Cream Mix. *Ind. Eng. Chem.*, **20**, 376—8 (1928).

Williams, O. E. Sandy Ice Cream. *N.Y. Produce Review*, **53**, 878—84 (1922).

Williams, O. E., and Peter, P. N. A New Form of Lactose Crystal Found in Sandy Ice Cream. *J. Dairy Sci.*, **13**, 471—7 (1930).

Zoller, H. F. Separation of Ice in Freezing Ice Cream. *Ice Cream Trade J.*, **17**, (9) 45—7 ; (10) 50—2 (1921) ; Rotating Thermocouple and Cold Junction for Temperature Studies in Commercial Ice Cream Machines. *Ibid.*, **17** (8) 40—3 (1921).

COLLOID PROBLEMS IN INFANT FEEDING

Alexander, J. Some Novel Aspects of Colloidal Protection. *Ind. Eng. Chem.*, **15**, 283—5 (1923) ; Colloidal Protection. *Chem. Ind.*, **43**, 1256—8 (1924).

Alleman, O. The Coagulation of Milk and the Physical Properties of the Curd. *Kolloid-Z.*, **24**, 27—42 (1919).

Arloing, F., and Delore, P. Decaseinated Milk and Digestive Anaphylaxis. *Compt. rend. soc. biol.*, **99**, 1987—9 (1930).

Arnold, L. Influence of Acidified Milk on Duodenal Function in Infants. *Am. J. Diseases Children*, **31**, 668—75 (1926).

Arthus. The Digestion of Milk. *Arch. intern. physiol.*, **18**, 133—46 (1921).

Aschenheim, E., and Stern, G. The Influence of Various Carbohydrates on the Coagulation of Milk. *Biochem. Z.*, **102**, 98—123 (1920).

Beal, G. D., and Neff, A. Gelatin in Medicine. *J. Am. Pharm. Assocn.*, **19**, 261—5 (1928).

Bell, M. The Composition of Human Milk. *J. Biol. Chem.*, **80**, 239—47 (1928).

Bergheim, O., *et al.* The Gastric Response to Foods : (2) A Fractional Study of the Coagulation of Milk in the Human Stomach. *Am. J. Physiol.*, **48**, 411—8 (1919).

Blenkle, V. A. Protein Milks in Infant Feeding. *Arch. Pediatrics*, **42**, 743—60 (1925).

Bosworth, A. W. Human Milk. *J. Biol. Chem.*, **20**, 707—9 (1915).

Bosworth, A. W., and Bowditch, H. I. Studies of Infant Feeding. The Chemical Changes Produced by the Addition of Lime Water to Milk. *J. Biol. Chem.*, **28**, 431—5 (1917).

Bosworth, A. W. The Casein of Cow Milk and Human Milk in their Relation to Infant Feeding. *Am. J. Diseases Children*, **22**, 193—201 (1921).

Brennemann, J. Boiled *v.* Raw Milk : an Experimental Study of Milk Coagulation in the Stomach, together with Clinical Observations on the Use of Raw and Boiled Milk. *J. Am. Med. Assocn.*, **60**, 575—82 (1913) ; **67**, 1413—19 (1916) ; Coagulation of Cow Milk in the Human Stomach. *Arch. Pediatrics*, **34**, 81—117 (1917).

Buckley, S. S. The Physical Character of the Curd of Milk from Different Breeds ; Curd as an Index of the Food Value of Milk ; Studies of the Proteid Contents of Milk. Md. Agr. Expt. Sta., *Bull.*, **184** (1914).

Burg, B. van der. The Composition, Specific Gravity and pH of Colostrum. *Nederland. Tijdschr. Hyg. Microbiol. Serol.*, **5**, 56—65 (1930).

Chasseray, E. Clinical Dietetics of Infancy : Buttermilk, Food and Medicine. *Rev. prat. malad. pays chauds*, **4**, 1241—70 (1925).

Clark, W. M. The Reaction of Cow Milk Modified for Infant Feeding. *J. Med. Research*, **31**, 431—53 (1915).

Courtney, A. M. Some Differences in the Behaviour of Raw, Pasteurised, Boiled, Evaporated and Dried Milk at the Hydrogen Ion Concentration of the Stomach. *Canad. Med. Assocn. J.*, **17**, 919—22 (1927).

Courtney, A. N., and Brown, A. The Protein and Non-Protein Fractions of Some Samples of Woman's Milk. *Arch. Diseases Childhood*, **5**, 36—41 (1930).

Denis, W., and Talbot, F. B. A Study of the Lactose, Fat and Protein Content of Women's Milk. *Am. J. Diseases Children*, **18**, 93—100 (1919).

Dennet, R. H. The Use of Boiled Milk in Infant Feeding. *J. Am. Med. Assocn.*, **63**, 1991—5 (1914).

Dunham, B. S. Acidification of Milk with Vinegar (Acetic Milk) in Infant Feeding. *Am. J. Diseases Children*, **29**, 200—5 (1925).

Eckhardt, R. A. Thick Cereal Formulas and Butter-Flour Mixtures in Infant Feeding. *Arch. Pediatrics*, **42**, 761—6 (1925).

Elsdon, G. D. Human Milk, *Analyst*, **41**, 74 (1916).

Elterich, T. O., Boyd, D. H., and Neff, A. Use of Gelatin as a Supplemental Food in the Infant's Dietary : An Historical Summary and the Report of a Study. *Arch. Pediatrics*, **47**, 286—319 (1930).

Faber, H. K. Hydrochloric Acid Milk in Infant Feeding. *Am. J. Diseases Children*, **26**, 401—10 (1923) ; Acidified Milks. *Ibid.*, **31**, 395—409 (1926).

Ferrier, O., and Chenard, V. Practical Method of Modifying Cow or Goat Milk to be Used in Place of Human Milk. *J. pharm. chim.*, **6**, 562—3 (1927).

Findlay, L., *et al.* Discussion : Is the Modification of Cow Milk Necessary in Infant Feeding ? *Proc. Roy. Soc. Med.*, **18**, *Sect. Diseases Children*, 73—83 (1925).

Fischer, A. Cow Milk and Vegetable Milk : Their Differences in the Gastric Digestion with Special Reference to the Problem of Cow Milk Intolerance. An Experimental Dietetic Study. *Arch. Verdauungs-Krankh.*, **20**, 13—48 (1914).

Flood, R. G. Acid Effect of Hydrochloric Acid and Lactic Acid Milk. *Am. J. Diseases Children*, **29**, 757—60 (1925).

Freudenberg, E. Physiology of Digestion in the Infant. *Monatschr. Kinderheilk*, **38**, 101—8 (1928).

Gabathuler, A. Digestion of Proteins of Different Milks by Pepsin-HCl and Pancreatin, With or Without the Presence of Electrolytes and Non-Electrolytes. *Fermentforschung*, **3**, 81—192 (1920).

Gellhorn, W. Casein Milk Feeding in Infancy and Childhood. *J. Am. Med. Assocn.*, **65**, 953—5 (1915).

Gleich, M. Lactic Acid Milk in the Feeding of Premature Children. *Arch. Pediatrics*, **42**, 432—9 (1925).

György, P. The Acidity of the Stomach of the Infant. *Arch. Kinderheilk.*, **72**, 1—5 (1922).

Hawk, P. B. Use of Gelatin in Milk for Feeding. *Proc. Soc. Exptl. Biol. Med.*, **20**, 269—70 (1923).

Hekma, E. The Casein Particles Suspended in Milk (Milk Platelets). *Verslag. Land. Onderzoek. Rijkslandbouwproefsta.*, **27**, 13—22 (1922).

Hess, A. F., and Matner, M. J. Value of Milk Acidified with Lemon Juice. Its Combination with Egg Yolk to Add the Anti-rachitic Factor. *J. Am. Med. Assocn.*, **82**, 1604—6 (1924).

Hess, J. H., and Chamberlain, I. McK. Gelatin Added to the Diet of Artificially-Fed Infants. *Ibid.*, **89**, 1423—6 (1927).

Hill, R. L. A Test for Determining the Character of the Curd from Cow Milk and its Application to the Study of Curd Variance as an Index to the Food Value of Milk for Infants. *J. Dairy Sci.*, **6**, 509—26 (1923) ; The Physical Curd Character of Milk and its Relationship to the Digestibility and Food Value of Milk for Infants. Utah Agr. Expt. Sta., *Bull.*, **207**, 1—32 (1928).

Holt, L. E., *et al.* Studies in Infant Metabolism and Nutrition : The Composition and Preparation of Protein Milk (Eiweissmilch). *Am. J. Diseases Children*, **10**, 172—82 (1915).

Johnson, C. K. The Use of Acidified Milk in Infant Feeding. *Boston Med. Surg. J.*, **193**, 1231—2 (1925).

Kohn, F. G. Differentiation of Cow and Goat Milk. *Z. Fleisch. Milchhyg.*, **39**, 279—81 (1929).

Krauss, F. I. Acidified Milk in Infant Feeding. *J. Med. Soc. New Jersey*, **25**, 792—7 (1928).

Leary, J. T., and Sheib, S. H. The Influence of the Coagulation by Rennin Upon the Gastric Digestion of Milk Proteins. *J. Biol. Chem.*, **28**, 393—8 (1917).

Lepine, V. E. Differences between Cow Milk and Human Milk. *Arch. Pediatrics*, **43**, 353—79 (1926).

Loebenstein, F. Types of Casein Coagulation. *Kolloid-Z.*, **32**, 264—72 (1923) ; **34**, 227—30 (1924).

Lowenfeld, M. F., *et al.* A Study of the Variations in the Chemical Composition of Normal Human Colostrum and Early Milk. *Biochem. J.*, **21**, 1—15 (1927).

Mai, H., and Keller, A. The Differentiation of Human and Cow Milk. *Klin. Wochschr.*, **9**, 535—6 (1930).

McCollum, E. V. The Significance of Colloids in the Dietary. Bogue's "Theory and Application of Colloidal Behaviour." (New York, 1924), Vol. 2, 683—99.

McLachlan, T. The Manufacture of Infants' Foods. *Food Manfr.*, **6**, 65—71 (1931).

Meigs, E. B., and Marsh, H. L. The Comparative Composition of Human Milk and of Cow Milk. *J. Biol. Chem.*, **16**, 147—68 (1913).

Mrozek, O., and Schlag, H. The Colostrum of Cow Milk *Milchwirtschaft. Forsch.*, **4**, 183—210 (1927).

Müller, E., and Schloss, E. Adaptation of Cow Milk to Resemble Human Milk for the Purpose of Infant Nutrition. An Historical Critical Study. *Jahrb. Kinderheilk.*, **80**, 42—64 (1914).

Myers, B. An Investigation of the Analysis of Human Milk. *Brit. J. Diseases Childhood*, **24**, 249—57 (1927).

Nakamura, M. The Quantitative Analysis of Woman's Colostrum. *Mitt. med. Fak. Kais. Univ. Tokyo*, **32**, 235—50 (1924) ; Physico-Chemical Studies of Woman's Milk. *Kyoto Ikadaigaku Zasshi*, **1**, 69—70, 1003—34 (1927).

Neff, A. Exclusive Milk and Gelatinised Milk Diets. *Med. J. Record*, **128**, 67—8 (1928).

Neukirch, H. Value of Protein Milk as a Medicinal Food. *J. praticiens*, **39**, 602 (1925) ; *Bull. soc. hyg. aliment.*, **13**, 527 (1925).

Orla-Jensen, and Spur, B. The Digestibility of Fresh and Soured Cow Milk. *Lait*, **4**, 845—8 (1924).

Perlman, H. H. Comparative Value of Gelatin as an Accessory Infant Food. *Arch. Pediatrics*, **45**, 14—20 (1928).

Porcher, C. Influence of Acidity on Clot Formation of Milk. *Rev. gén. coll.*, **7**, 260—70 (1929).

Provan, A. L. Studies on Colostrum. *Welsh J. Agr.*, **4**, 141—7 (1928).

Remington, J. S. Breakfast, Invalid and Infant Foods. *Ind. Chemist*, **3**, 155—7 (1927).

Rice, F. E. Infant Stomachs and Colloid Chemistry. *J. Elisha Mitchell Sci. Soc.*, **43**, 20—1 (1927) ; Use of Unsweetened Evaporated Milk in Infant Feeding. *Arch. Pediatrics*, **44**, 758—65 (1927).

Rosenstern, J., and Lauter. Investigations on the Importance of the State of Solution, Especially the Degree of Dispersion of Organic Foods in Infant Feeding (Crystalloids, Colloids, Suspensions, Emulsions). *Z. Kinderheilk*, **32**, 316—21 (1922).

Spolverini, I. Investigations on the Physico-Chemical Colloids of Milk and their Consequence in the Practical Nutrition of Infants. *Lait*, **10**, 21—32 (1930).

Tadokoro, T., and Sato, S. Studies on the Protective Colloids of Milk. *J. Biochem. Japan*, **1**, 433—43 (1922).

Terry, R. W. Chemistry of Milk Curd Modification. *Arch. Pediatrics*, **38**, 465—90 (1920).

Wallen-Lawrence, Z., and Koch, F. C. The Relative Digestibility of Unsweetened Evaporated Milk, Boiled Milk and Raw Milk by Trypsin in Vitro. *Am. J. Diseases Children*, **39**, 18—33 (1930).

Wardlaw, H. S. H. Composition of Human Milk in Australia. *J. Proc. Roy. Soc. N.S. Wales*, **49**, 169—98 (1915).

Weeks, V. T. Survey of Acid Milk in Infant Feeding. *Arch. Pediatrics*, **42**, 722—34 (1925).

Weissenberg, H. Citric Acid-Whole Milk, a New Food for Sick and Healthy Infants. *Arch. Kinderheilk.*, **78**, 276—301 (1926).

Ylppö, A. Composition of Reindeer Milk and its Use as an Infant Food. *Z. Kinderheilk.*, **43**, 255—7 (1927).

VITAMINS

Dogadkin, B., and Yanovska, B. I. Colour Reactions of Vitamin C : (1) Dispersion and Behaviour with Different Adsorbents of Substances Specific for Bezssonoff's Reagent. *Biochem. Z.*, **213**, 86—94 (1929).

Grafe, V., and Magistris, H. The Relation between Vitamin Effect and Capillary Activity of Phosphatides. *Biochem. Z.*, **177**, 16—26 (1926).

Hahn, F. V. von. Colloid Aspects of the Problem of Vitamins. *Kolloid-Z.*, **36**, 271—4 (1925) ; Colloid Chemical Studies on Surface Activity and Vitamin Action : (1) The Vitaminoid Condition. *Pflüger's Arch.*, **208**, 732—44 (1925) ; (2) Surface Activity and the Vitamin Content of Foods, *Ibid.*, **208**, 745—60 (1925) ; Influence of Surface-Active Substances on the Fungus Growths in Thrush in Respect to Water-Soluble Vitamins. *Kolloid-Z.*, **40**, 321—7 (1926) ; Colloidobiological Study of the Vitamins. Alexander's "Colloid Chemistry," Vol. 2, 917—30 (New York, 1928) ; Review of the Vitamin Question. *Verhandl. Naturwiss. Ver. Hamburg.*, **3**, 11—45 (1928) ; Vitamin Activity and Surface Tension Action. *Biochem. Z.*, **222**, 259—71 (1930).

Hahn, F. V. von, and Junker, H. Colloid Biological Studies on Thrush. *Jahrb. Kinderheilk.*, **121**, 85—100 (1928).

Salmon, W. D., Guerrant, N. B., and Hays, I. M. The Effect of Hydrogen Ion Concentration upon the Adsorption of the Active Factors of Vitamin B Complex by Fuller's Earth. *J. Biol. Chem.*, **80**, 91—101 (1928).

Schepilewskaya, N. E. Vitamin Action and Surface Activity : (1) Tests with Compounds having Surface Activity in Avitaminosis of Guinea Pigs. *Biochem. Z.*, **204**, 371—88 (1929) ; (2) Anti-scorbutic Action of Certain Plant Juices and their Surface Tension Activity. *Ibid.*, **208**, 334—51 (1929) ; Parallelism between the Changes of Antiscorbutic Action and the Surface Activity of Cabbage Juice. *Ibid.*, **210**, 334—7 (1929).

SUGAR

Allen, A. W. Manufacture of Refined White Sugar. *Chem. Met. Eng.*, **32**, 352—60 (1925).

Dědek, J., and Nováček, J. The Crystallisation of Sucrose Solutions. *Kolloid-Z.*, **42**, 163—7 (1927).

Deerr, N. The Clarification of Cane Juice in the Manufacture of Raw Sugar. *Intern. Sugar J.*, **18**, 502—6, 558—63 (1916).

Guillaume, J. Sugar Refining. *Bull. assocn. chim. sucr.*, **46**, 341—51 (1929).

Haan, J. S. de. What is Our Experience with the Different Methods for the Manufacture of White Sugar and What Method Suits the Commercial Situation ? *Arch. Suikerind.*, **25**, 475—551 (1917).

Hamous, J. The Diffusion of Non-Sugars into Sugar Juices. *Listy Cukrovar.*, **48**, 52 (1929).

Owen, B. J. Desiccation of Sugar Beet and the Extraction of Sugar. (London, H. M. Stationery Office. 1927).

Zerban, F. W. The Clarification of Cane Juice without Chemical Treatment. Louisiana Agr. Expt. Sta., *Bull.*, **173**, 26 pp. (1920).

COLLOID PROBLEMS IN SUGAR MANUFACTURE

Albritton, G. G., and Horton, P. M. Surface Tension. *Facts about Sugar*, **22**, 40—1 (1927).

Badollet, M. S., and Paine, H. S. Basic Dyes as Flocculating Agents for Approximate Quantitative Determination of Colloids in Sugar-House Liquors. *Intern. Sugar J.*, **28**, 23—8, 97—103, 137—40 (1926); Revised Directions for Operation of the Dye Test for Approximate Determination of Colloid Materials in Sugar and Sugar Liquors. *Planter Sugar Mfr.*, **79**, 121—3 (1927).

Bardorf, C. F. Migration of Cane-Wax Complex through Stations of a Refinery. *Ind. Eng. Chem.*, **20**, 258—61 (1928); Cane-Wax Complex in Juices from Cane-Sugar Mills. *Ibid.*, **21**, 366—8 (1929).

Bergé, J. *Apropos* the Electrolysis of Sugar Juices. *Sucr. Belge.*, **47**, 283—5; 302—4 (1928); Colloid Chemistry in its Relation to the Sugar Industry. *Bull. soc. chim. Belg.*, **38**, 31—46 (1929).

Bird, M. The Removal of Gums by Various Methods of Clarification. *Intern. Sugar J.*, **30**, 196—7 (1928).

Carrick, M. S. Some Studies in Fondant Making. *J. Phys. Chem.*, **23**, 589—602 (1919).

Claassen, H. Frothing of Boiling Liquids, Foam Separators and Juice Traps in Sugar Factories. *Zentr. Zuckerind.*, **27**, 500—2 (1919).

Dědek, J. Colloid Chemistry in the Sugar Industry. *Listy Cukrovar*, **39**, 185—90, 193—6 (1921) *Z. Zuckerind. cechoslovak. Rep.*, **45**, 251—3, 263—6, 275—8 (1921).

Dědek, J., and Nováček, J. Surface Tension and Adsorbents. *Sugar*, **30**, 99—101, 149—51, 199—200, 247—8 (1928).

Farnell, R. G. W. The Effect of Cold and Hot Liming on the Removal of Albumin from Cane Juices. *Intern. Sugar J.*, **26**, 359—63 (1924); Report on a Preliminary Investigation of the Colloids of Cane Juices and Molasses. *Ibid.*, **26**, 420—8 (1924); The Pectin and Pentosan Constant of Cane Juices, Sirups and Molasses and its Removal. *Ibid.*, **26**, 480—6 (1924); The *Rôle* of Colloids in Cane-Sugar Manufacture. *Ibid.*, **27**, 94—5 (1925); Investigation of the Colloids of Cane Juices and Sirups in Natal and Mauritius. *Ibid.*, **27**, 254—60 (1925).

Fischer, M. H., and Hooker, M. O. The Colloid Chemistry of Fehling's Test. *Science*, **45**, 505—7 (1917); Note on the Colloid Chemistry of Fehling's Sugar Test. *Intern. Sugar J.*, **21**, 76—8 (1919).

Haddon, E. Influence of Starch on the Manufacture of Sugar and its Removal from the Juice. *Rev. agr. Maurice*, **5**, 80—2 (1928).

Hardy, F. The Components of Sugar-Cane Juice. Their Mode of Occurrence and Behaviour during Sugar Manufacture. *Tropical Agr. (Trinidad)*, **4**, (Suppl. 9), 38—40 (1927).

Honig, P. Determination and Significance of Surface Tension of Sugar Solutions. *Chem. Weekblad.*, **23**, 265—9 (1926); Colloid-Chemical Research Work in the Sugar Industry in Recent Years. *Arch. Suikerind.*, **36**, 591—612 (1928).

Kruyt, H. R. The Significance of Recent Developments in Colloid Chemistry. *Arch. Suikerind.*, **29**, 1102—6 (1921).

Kutzev, S. S., and Kotlyarenko, M. P. Investigation on the Determination of Surface Tension in Raw and Refinery Sugar Products. *Zhurnal Sakharnoi. Prom.*, **2**, 357—61 (1928); The Phenomenon of the Colloidal State of Substances in the Products of Beet Sugar Manufacture. *Ibid.*, **3**, 297—306 (1929).

Lindfors, K. R. Surface Tension of Sugar-Factory Products. *Ind. Eng. Chem.*, **16**, 813—6 (1924); Physical Examination of Sugar Juices. *Ibid.*, **17**, 1155—6 (1925).

Paine, H. S., Badollet, M. S., and Keane, J. C. Colloids in Cane and Beet Sugar Manufacture. *Ind. Eng. Chem.*, **16**, 1252—8 (1924).

Paine, H. S., and Badollet, M. S. The Isoelectric Point Determination in Corn Sugar and Corn Sirup Manufacture. *Facts About Sugar*, **21**, 1212—3 (1926); Adsorption of Raw Sugar Colloids by Bone Char. *Planter Sugar Mfr.*, **79**, 21—3 (1927); Colloid Elimination during Bone Char Filtration in Cane Sugar Refineries. *Facts About Sugar*, **22**, 62—4 (1927).

Paine, H. S., and Balch, R. T. Nature and Distribution of the Non-Sugars affecting the Quality of White Beet Granulated Sugars. *Intern. Sugar J.*, **28**, 472—7 (1926).

Prasad, M., Shrivastava, D. L., and Gupta, R. S. The Mechanism of the Adsorption of Sugars by Colloidal Solutions and Precipitates. *Kolloid-Z.*, **37**, 101—4 (1925).

Prinsen-Geerligs, H. C. Modern Views on Juice Clarification. *Arch. Suikerind.*, **30**, 877—8 (1922).

Rohland, P., and Meysahn, V. Method for Determining Colloids in Waste Water Containing Sugar. *Z. ver deut. Zuckerind.*, **63**,167 (1913).

Rose, E. F. O. The Filtration of Liquids, especially of Juices in the Cane Sugar Industry. *Arch. Suikerind.*, **32**, 527—37 (1924).

Ruff, H. T., and Withrow, J. R. The Determination of Gums in Sugar Products. *Ind. Eng. Chem.*, **14**, 1131—4 (1922).

Saillard, E. Adsorption in the Sugar Industry. *Compt. rend.*, **190**, 1518—20 (1930).

Sazavsky, V. Surface Tension of Sugar Factory Juices. *Z. Zuckerind. cechoslovak. Rep.*, **50**, 378—80 (1926).

Seip, J. J. Colloids in their Relation to Clarification. *Sugar News*, **9**, 81—6 (1928).

Stanek, V. The Action of Lime on the Non-Sugars Precipitated from Diffusion Juice by Carbonation. *Z. Zuckerind. cechoslovak. Rep.*, **48**, 301—7 (1924).

Weimarn, P. P. V. Colloidal Sugar. *Jap. J. Chem.*, **2**, 63—72 (1925). *Kolloid-Z.*, **36**, 176—7 (1925).

Wells, C. H. A Colloidal Method of Cane-Juice Clarification. *Louisiana Planter*, **71**, 394—5 (1923).

Zieminski, S. Electrodialytic Demineralisation of Sucrose Solutions. *Przemysl Chem.*, **13**, 429—45 (1929).

JUICE PURIFICATION

Aten, A. H. W. Carbonation of Sugar-Lime Solutions. *Rec. trav. chim.*, **45**, 753—71 (1926) ; The pH of Carbonation Process in the Manufacture of Sugar and the Properties of Albuminoids Present. *Ibid.*, **45**, 792—802 (1926).

Balch, R. T., and Keane, J. C. Automatic Control of the Carbonation Process in Beet-Sugar Manufacture. *Ind. Eng. Chem.*, **20**, 1148—51 (1928).

Bond, J. D. Defecation in Cane Sugar Manufacture. *Ind. Eng. Chem.*, **17**, 492—5 (1925).

Brewster, J. F. Cane Juice Clarification—Theory and Practice *Louisiana Planter Ref. Book*, **2**, 55—7 (1924).

Dale, J. K., and Hudson, C. S. Sugar-Cane Juice Clarification for Sirup Manufacture. U.S. Dept. Agr., *Bull.*, **921**, 1—15 (1920).

Dědek, J. Solutions of Sucrose and Calcium. *Listy Cukrovar.*, **48**, 403—11 (1930).

Farnell, R. G. W. The Precipitation of Calcium Sulphite under Various Conditions with Special Reference to Sugar Factories. *J. Soc. Chem. Ind.*, **44**, 530—8 T (1925) ; The Precipitation of Calcium Phosphate under Various Conditions with Special Reference to Sugar Factories. *Ibid.*, **45**, 343—7 T (1926).

Friedrich, H. Purifying Diffusion Juices by Means of Small Quantities of Lime at Temperatures over 100° C. *Z. Zuckerind. cechoslovak. Rep.*, **54**, 34—6 (1929).

Harloff, W. H. T. Modern Juice Purification. *Arch. Suikerind.*, **28**, 802—24 (1920).

Hartmann, E. E. Pêtree Process for the Clarification of Cane Juices. *Ind. Eng. Chem.*, **16**, 1178—80 (1924).

Hines, C. W. Cane Juice Clarification. *Sugar*, **17**, (7), 36—9 (1915) ; *Philippine Agr. Rev.*, **9**, 339—47 (1916).

Horne, W. D. Recent Advances in Defecation. *Ind. Eng. Chem.*, **12**, 1179—80 (1920).

Horne, W. D. Super-Defecation of Cane Juice. *Ibid.*, **16**, 732—3 (1924).

Jagt, H. A. C. van de. Chemical and Physical De-Liming of Sugar Solutions and Sirups. *Chem. Weekblad.*, **23**, 566—72 (1926).

Konn, V. Physico-Chemical Changes in Sugar During Clarification. *Listy Cukrovar.*, **48**, 105—16 (1929) ; *Z. Zuckerind. cechoslovak. Rep.*, **54**, 173—86 (1930).

Levy, N. A New Method of Purifying Sugar Juices. *Bull. assocn. chim. sucr. dist.*, **41**, 224—6 (1923).

Pachlopnik, F. Precipitation of Acids during Defecation and Carbonation. *Z. Zuckerind. cechoslovak. Rep.*, **50**, 269—76, 281—8 (1926).

Paine, H. S., Keane, J. C., and McCalip, M. A. Influence of Phosphate and Colloid Contents of Cane Juice on Defecation. *Ind. Eng. Chem.*, **20**, 262—7 (1928).

Peck, S. S. A New Conception of the Theory of the Clarification of Cane Juice. *Intern. Sugar J.*, **21**, 70—2 (1919).

Roxas, M. L. Clarification in Raw Sugar Factories. *Intern. Sugar J.*, **26**, 486—91 (1924).

Waterman, H. I., and Aken, J. S. A. J. M. V. Precipitation of Sugar Solutions with Lime. Contribution to the Knowledge of the Steffen Process for the Manufacture of Sugar from Molasses. *J. Soc. Chem. Ind.*, **46**, 411—3 T (1927).

pH CONTROL

Aten, A. H. pH of Carbonation Process in the Manufacture of Sugar and Properties of the Proteins Present. *Rec. trav. chim.*, **45**, 792—802 (1926).

Baissac, L. Determination of Hydrogen Ion Concentration in the Cane Sugar Industry. *Planter Sugar Manfr.*, **76**, 348—50 (1926).

Balch, R. T., and Paine, H. S. Factory Operation of Automatic Electrometric pH Control of Cane Juice Defecation. *Ind. Eng. Chem.*, **20**, 348—53 (1928).

Barbaudy. Control of pH in the Sugar Industry. *Chimie et industrie*, **18**, 984—92 (1928).

Blowski, A. A., and Holven, A. L. Hydrogen Ion Concentration as a Basis of Refinery Alkalinity Control. *Ind. Eng. Chem.*, **17**, 1263—6 (1925).

Britton, H. T. S. The Importance of Hydrogen Ion Concentrations in Sugar Manufacture. *Ind. Chem.*, **3**, 508—11 (1927).

Hauge, S. M., and Willaman, J. J. Effect of pH on Adsorption by Carbon. *Ind. Eng. Chem.*, **19**, 943—53 (1927).

Honig P., and Khainovsky, V. Determination and Application of pH in Cane Sugar Manufacture. *Mededeel. Proefstat. Java Suikerind.*, No. **17**, 639—707 (1927).

Honig, P. The Real Acidity in Sugar Manufacture, its Determination with Indicators and its Effect on the Product. *Arch. Suikerind.*, **36**, 212—22 (1928).

Hurst, R. H. Some Applications of Physico-Chemical Determinations to the Control of Sugar Manufacture. *Trop. Agr. (Trinidad)*, **4**, Sugar Supplement, 32—3 (1927).

Paine, H. S., and Balch, R. T. Hydrogen Ion Concentration and the Defecation of Cane Juice. *Planter Sugar Manfr.*, **79**, 127—32, 148—50 (1927).

Perkins, H. Z. E. Hydrogen Ion Determination as a Method of Refinery Control. *Ind. Eng. Chem.*, **15**, 623—4 (1923).

THEORY OF ADSORPTION BY CARBON

Alekseevskii, E. V. The Chemical Theory of Adsorption Phenomena. *J. Russ. Phys. Chem. Soc.*, **62**, 817 (1930).

Alekseevskii, E. V., and Musakin, A. P. Influence of Some Chemical and Physical Factors on the Activation of Carbon. *Ibid.*, **62,** 205—19 (1930).

Ardagh, E. G. Activated Carbon. *J. Soc. Chem. Ind.*, **40,** 230—3 T (1921).

Bardorf, C. F. Notes on Some Decolorising Blacks. *Can. Chem. J.*, **4,** 207—11 (1920).

Barker, M. E. Mechanism of Charcoal Activation. *Ind. Eng. Chem.*, **22,** 926—31 (1930).

Bartell, F. E., and Miller, E. J. Adsorption by Activated Sugar Charcoal. *J. Am. Chem. Soc.*, **44,** 1866—80 (1922).

Bartell, F. E., and Fu, Y. The Specific Surface Area of Activated Carbon and Silica. *Am. Coll. Symp. Monog.*, **7,** 135—49 (1930).

Berl, E., and Burkhardt, H. Preparation of Active Carbon. *Z. angew. Chem.*, **43,** 330—3 (1930).

Blowski, A. A., and Bon, J. H. Decolorising Carbons. *Ind. Eng. Chem.*, **18,** 32—42 (1926). See the correspondence on this paper, *ibid.*, p. 649.

Boutaric, A. Formulas Representing Adsorption Isotherms. *J. chim. phys.*, **26,** 492—6 (1929).

Chaney, N. K. The Activation of Charcoal. *Trans. Am. Electrochem. Soc.*, **36,** 91—111 (1919).

Chaney, N. K., Ray, A. B., and John, A. St. The Properties of Activated Carbon which Determine its Industrial Applications. *Ind. Eng. Chem.*, **15,** 1244—55 (1923).

Chufarov, G. I. The Theory of the Activation of Carbon. *J. Russ. Phys. Chem. Soc.*, **62,** 883—95 (1930).

Dědek, J. Estimation of the Value of Activated Carbons. *Sugar*, **29,** 255—6, 307—9 (1927); *Intern. Sugar J.*, **29,** 446—7 (1928).

Edelstein, V. Influences having a Disturbing Influence on the Analytical Determination of the Decolorising Power of Active Carbon. *Z. Zuckerind. cechoslovak. Rep.*, **54,** 257—65 (1930).

Firth, J. B. The Sorption Activity of Carbon. *J. Soc. Chem. Ind.*, **42,** 242—4 T (1923).

Honig, P. Comparative Study of Active Carbons. *Kolloid-chem. Beihefte*, **22,** 345—420 (1926); The Production and Composition of Decolorising Carbons. *Chem.-Ztg.*, **52,** 7—8, 34—5 (1928).

Horne, W. D. United States Patents on Bone Black and Decolorising Carbons. *Ind. Eng. Chem.*, **16,** 670 (1924).

Horton, P. M., and Sengson, P. T. Comparative Ash Adsorption of Vegetable and Bone Chars. *Ind. Eng. Chem*, **16,** 165—7 (1924).

Knowles, H. I. Examination of Bone Black. *Ind. Eng. Chem.*, **19,** 222—5 (1927).

Kolthoff, I. M. Estimation of the Adsorbing Power of Carbon. *Pharm. Weekblad.*, **58,** 630—56 (1921).

Kroetz, C. The Adsorption of Hydrogen and Hydroxyl Ions by Animal Charcoal. *Biochem. Z.*, **153,** 173—84 (1924).

Lowry, H. H. On the Nature of Active Carbon. *J. Phys. Chem.*, **34,** 63—73 (1930).

McBain, J. W. Theories of Adsorption and the Technique of its Measurement. *Nature*, **117**, 550 (1926).

Mantell, C. L. "Industrial Carbon" (New York, 1928).

Mecklenburg, W. Active Carbon. Liesegang's "Kolloidchemische Technologie" (Dresden, 1931).

Ogawa, I. Adsorption Power and Electrical Behaviour of Different Preparations of Carbon. *Biochem. Z.*, **161**, 275—94 (1925); Further Studies of the Adsorption Capacity of Different Preparations of Carbon. *Ibid.*, **172**, 249—61 (1926).

Schilov, N., Dubinin, M., and Toporov, S. Mixed Adsorbents. *Kolloid-Z.*, **49**, 120—6 (1929); The Chemical State of the Surface of Active Charcoal. *Z. physik. Chem.*, **Abt. A. 149**, 211—21 (1930); The Surface Oxides of Active Charcoal. *Ibid.*, **150**, 31—6 (1930).

Sporry, E. Determining the Values of Decolorising Carbons. *Chem.-Ztg.*, **47**, 203—5 (1923).

Strachan, J. T. Activated Carbon: Some Industrial Applications. *J. Soc. Chem. Ind.*, **47**, *Chem. Ind.*, 1203—14 (1928).

Syrkin, J. K., and Bernstein, L. I. The Kinetics of Sorption. *Z. anorg. allegem. Chem.*, **152**, 105—12 (1926).

Tadokoro, T. Determination by the Adsorption Formula of the Minimum Quantity of Decoloriser Required for Perfect Decolorisation. *J. Chem. Ind. Japan*, **21**, 405—11 (1918).

Teeple, J. E., and Mahler, P. Testing Decolorising Carbons. *Ind. Eng. Chem.*, **16**, 498—500 (1924).

Těrechov, P., and Dědek, J. The Character of Active Carbons. *Sugar*, **30**, 349—50; 395—6, 443—4 (1928).

Traube, J. Adsorption Effects of Carbons. *Z. Ver. deut. Zuckerind.*, **77**, 355—64 (1927).

Turrentine, J. W., and Tanner, H. G. Potash from Kelp: The Applicability of Kelpchar as a Bleaching and Purifying Agent. *Ind. Eng. Chem.*, **14**, 19—24 (1922).

Vašátko, J. The Efficiency of Activated Charcoal. *Listy Cukrovar.*, **48**, 89—102 (1929).

Wayne, T. B. Some Adsorption Properties of Bone Char. *Ind. Eng. Chem.*, **20**, 933—40 (1928).

Wickenden, L., and Hassler, J. A Rapid Method for Comparing the Decolorising Efficiency of Charcoals. *Ind. Eng. Chem.*, **8**, 518—19 (1916).

USE OF CARBON IN SUGAR TECHNOLOGY

Badollet, M. S., and Paine H. S. Clarification of Starch Conversion Liquors in Manufacture of Corn Sugar and Corn Sirup. *Ind. Eng. Chem.*, **19**, 1245—6 (1927).

Bradley, A. B. Vegetable Decolorising Carbons. *J. Soc. Chem. Ind.*, **38**, 396—8 T (1919).

Brewster, J. F. A Review of the Literature on Decolorising Carbons. *Louisiana Planter*, **70**, 471—3 (1923).

Brewster, J. F., and Raines, W. G. The Effect of Some Decolorising Carbons upon the Colour and Colloids of Cane Juice. *Ind. Eng. Chem.*, **13**, 921—3 (1921); The Effect of Varying Hydrogen

Ion Concentration upon the Decolorisation of Cane Juice with Carbon. *Ibid.*, **13**, 1043—4 (1921).

Coates, C. E. Preparation and Evaluation of a Decolorising Char from Bagasse. *Ind. Eng. Chem.*, **14**, 295—8 (1922).

Dědek, J., and Tumova, B. Adsorption in Thick Juice by Layers of Carbon. *Z. Zuckerind. cechoslovak. Rep.*, **52**, 65—75 (1927).

Garino, M., and Benvenuto, G. Decolorising Carbons and Their Action on Molasses. *Bull. assocn. chim. sucr. dist.*, **46**, 326—31 (1929).

Honig, P., and Bogtstra, J. F. Measurement of Colour in the Sugar Industry. *Archief. III Meded.*, **1928**, No. 1, 1—35.

Horne, W. D. Colour and Ash Adsorption by Boneblack and Decolorising Carbons. *Ind. Eng. Chem.*, **14**, 1134—6 (1922).

Knowles, H. I. Temperature and Analytical Changes in Sugar Liquor during Bone Black Filtration. *Ind. Eng. Chem.*, **17**, 1151—3 (1925).

Rakusin, M. A. Comparative Adsorption of Sucrose and Caramel. *Chem.-Ztg.*, **46**, 770 (1922).

Rice, E. W., and Murray, G. W. Factors Influencing Char Filtration. *Ind. Eng. Chem.*, **20**, 276—7 (1928).

Sanders, M. T. Adsorption of Colour from Sugar Solutions by Chars. *Ind. Eng. Chem.*, **15**, 784—5 (1923) ; The Countercurrent Use of Decolorising Carbons. *Ibid.*, **20**, 791—4 (1928).

Sazavsky, V. Function of the Mineral Skeleton of Bone Char in Juice Purification. *Z. Zuckerind. cechoslovak. Rep.*, **52**, 13—20 (1927).

Spengler, O., and Landt, E. Comparative Experiments on the Adsorptive Power of Activated Carbons. *Z. Ver. deut. Zuckerind.*, **77**, 429—72 (1927).

Taegener, W. Purification of Potato and Maize Sirups by Activated Carbon. *Z. Spiritusind.*, **53**, 155—7 (1930).

Todt, F. Determination of the Effect of Decolorising Carbons on Sugar Juices by Means of Surface Tension Measurements. *Z. Ver. deut. Zuckerind.*, **76**, 253—72 (1926).

Vacha, J. Adsorption from Sugar Solutions : Adsorption of Sucrose by Activated Carbon. *Z. Zuckerind. cechoslovak. Rep.*, **55**, 123—7 (1930).

Vašátko, J. Adsorption of Sucrose by Active Charcoals. *Z. Zuckerind. cechoslovak. Rep.*, **52**, 21—32 (1927) ; Simultaneous Adsorption of Sucrose and Coloring Matter by Active Charcoals. *Ibid.*, **52**, 45—52 (1927) ; The Effect of Rate of Flow upon Decolorisation of Sugar Solutions with Activated Charcoal. *Listy Cukrovar.*, **48**, 137—42 (1929).

Wayne, T. B. Influence of Absorption Spectra of Technical Sugar Products on the Decolorising Efficiency of Bone Char. *Ind. Eng. Chem.*, **18**, 847—54 (1926).

Wiesner, J. The Decolorisation of Refinery Liquors with Active Carbons. *Z. Zuckerind. cechoslovak. Rep.*, **52**, 101—8, 120—3 (1927).

Wijnberg, A. The " Norit " Process of Manufacturing White Sugar. *Intern. Sugar J.*, **17**, 70—4 (1915).

Zamaron, J. Experiments on the Physico-Chemical Epuration of Sugar Products by Active Chars in the Presence of Sulphurous and Phosphoric Acids. *Bull. assocn. chim. sucr. dist.*, **47**, 259—63 (1930).

Zerban, F. W. Vegetable Decolorising Carbons and Their Use in the Cane Sugar Industry. Louisiana Agr. Expt. Sta., *Bull.*, **161** (1918); Studies in the Preparation of Vegetable Decolorising Carbons for the Cane Sugar Industry. *Ibid.*, *Bull.*, **167** (1919); The Relative Importance of Some Colouring Matters in Cane Juices and Sirups. *Ind. Eng. Chem.*, **12**, 744—51 (1920).

Zwet, J. K. Van Der. Influence of Activated Carbons on the pH and the Inversion of Sugar Solutions. *Zentr. Zuckerind.*, **34**, 1119—20 (1926).

COLLOID IMPURITIES IN SUGAR PRODUCTS

Brodowski, A. V. Colloids in Molasses. *Kolloidchem. Beihefte*, **29**, 261—353 (1929).

Dědek, J. Formation and Nature of Molasses. *Chem. Listy.*, **21**, 96—101, 163—70 (1927); *Z. Ver. deut. Zuckerind.*, **77**, 495—558 (1927).

Farnell, R. G. W. Report on a Preliminary Investigation of the Colloids of Cane Juices and Molasses. *Intern. Sugar J.*, **26**, 420—8 (1924).

Gundermann, E. The Precipitation of Colloids from Beet-Sugar Molasses. *Chem.-Ztg.*, **53**, 305—7, 322—3 (1929).

Helderman, W. D., and Khainovsky, V. The Effect of Colloids on the Viscosity of Our Java Sugar Cane Molasses. *Arch. Suikerind.*, **29**, 1229—35 (1921).

Genin, G. Dialysis of Molasses. *L'Industrie chimique*, **17**, 542—4 (1930).

Kameyama, N., and Kato, J. Electrodialysis of Exhausted Molasses. *J. Soc. Chem. Ind. Japan*, **31**, 201—2B (1928).

Khainovsky, V. The Effect of Colloids on the Viscosity of Sugar Cane Molasses. *Arch. Suikerind.*, **29**, 1344—7 (1921).

Lothrop, R. E., and Paine, H. S. Some Properties of Honey Colloids and the Removal of Colloids from Honey with Bentonite. *Ind. Eng. Chem.*, **23**, 328—32 (1931).

Parisi, E. Nitrogenous Substances of Beet Molasses. *Ann. chim. applicata.*, **15**, 555—68 (1925).

DECOLORISING EARTHS

Calvert, R. "Diatomaceous Earth" (New York, 1930), Chap. 7.

Devos, P. Affining of Sugars and Use of a Decolorising Earth in Sugar Factories. *Bull. assocn. chim. sucr. dist.*, **46**, 292—301 (1929).

Eckart, O. Fuller's Earth. *Z. angew Chem.*, **42**, 939—41 (1929).

Elsenbast, A., Elliott, R. D, and Sullivan, E. J. Use of Super-Cel in the Sugar Refining Industry. *Ind. Eng. Chem.*, **21**, 676—84 (1929).

Hardin, G. H., and Zerban, F. W. Adsorption Effects of Filtering Materials on Sugar Solutions. *Ind. Eng. Chem.*, **16**, 1175—7 (1924).

Paine, H. S., and Walton, C. F. Use of Kieselguhr in the Clarification of Cane Juice. *Science*, **53**, 266—7 (1921).

Scholz, A. Bleaching Earths of High Activity. *Chem.-Ztg.*, **53**, 899 (1929).

CARAMEL

Beal, G. D., and Zoller, H. F. The Preparation of Pure Surcose and Dextrose Caramels. *J. Am. Pharm. Assocn.*, **3**, 495—7 (1914).

Beal, G. D., and Applegate, G. The Preparation of Sucrose Caramel. *Ibid.*, **12**, 850—3 (1923).

Beal, G. D., and Bowey, D. F., The Preparation of Acid-Fast Caramels. *Ibid.*, **12**, 495—510 (1923).

Cunningham, M., and Doree, C. Chemistry of Caramel : Caramelan. *J. Chem. Soc.*, **111**, 589—608 (1917).

Garino-Canina, E. Caramel. *Giorn. vinicolo ital.* (Oct., 1926) ; *Notiz. chim. ind.*, **2**, 133—6 (1927).

Garino, M., and Tosonotti, A. Coloring Matters Formed by the Caramelisation of Sucrose and by the Action of Lime on Invert Sugar. *Giorn. chim. ind. applicata.*, **11**, 8—13 (1929).

Handcock, W. A. Notes on Caramel. *Brewers J.*, **51**, 167—9 (1915).

Kauffman, M. A Critical Study of Methods for the Determination of Caramel in Sugar Factory Products. *Arch. Suikerind.*, **28**, 2027—42 (1920).

Ling, A. R., and Nanji, D. R. The Action of Ammonia and of Amino Compounds on the Reducing Sugars : The Action of Ammonia on Dextrose and Laevulose. *J. Soc. Chem. Ind.* **41**, 151—5T (1922).

Lowitz, F. The Caramel Malt Question. *Allgem. Brauer-Hopfenztg.*, **55**, 823 (1915).

Rakuzin, M. A., and Nesmeianov, A. N. Change in Optical Activity of Sucrose on Heating. *Z. Untersuch. Lebensm.*, **48**, 151—2 (1924).

Ripp, B. Formation of Caramel Substances in the Presence of Nitrogenous Compounds. *Z. Ver. deut. Zuckerind.*, **76**, 627—55 (1926).

Sangiorgi, G. Caramelisation of Sugar and Commercial Caramel. *Giorn. farm. chim.*, **62**, 256—61 (1914).

Simmich, H. Commercial Caramels and Molasses Colouring Substances. *Z. Ver. deut. Zuckerind.*, **76**, 1—18 (1926).

PECTIN AND FRUIT JELLIES

Ahmann, C. F., and Hooker, H. D. The Estimation of Pectin and a Study of the Constitution of Pectin. Missouri Agr. Expt. Sta., *Res. Bull.*, **77**, 5—39 (1925) ; Determination of Pectin. *Ind. Eng. Chem.*, **18**, 412—4 (1926).

Ajou, G. The Determination of Pectin. *Giorn. chim. ind. applicata*, **9**, 454 (1927); Some Analytical Results with Lemons. *Riv. ital. essenze profumi.*, **11**, 107—11 (1929).

Angerhausen, J. The Juice of Rhubarb Stems. *Z. Untersuch. Lebensm.*, **39**, 122—30 (1920).

Appleman, C. O., and Conrad, C. M. Pectic Constituents of Peaches and their Relation to the Softening of the Fruit. Maryland Agr. Expt. Sta., *Bull.*, **283**, 1—8 (1926); The Pectic Constituents of Tomatoes and their Relation to the Canned Product. *Ibid., Bull.*, **291**, 1—17 (1928).

Arnon, C. Water in Jams, Jellies and Marmalades. *Ann. fals.*, **9**, 202—5 (1916).

Baker, G. L. Jelly Strength of Pectin Jells. *Ind. Eng. Chem.*, **18**, 89—93 (1926).

Barker, B. T. P. Cider Apple Jelly. *J. Soc. Chem. Ind.*, **37**, 243—6 T (1918).

Bell, J. C., and Wiegand, E. H. Extraction and Clarification of Pectinous Fruit Juices. Oregon Agr. Expt. Sta., *Circ.*, **63**, 4—12 (1925).

Beythien, A. Principles for the Evaluation of Marmalades, Confections, Jams and Jellies. *Z. Untersuch. Lebensm.*, **56**, 80—106 (1928).

Bigelow, W. D., and Dunbar, P. B. The Acid Content of Fruits. *Ind. Eng. Chem.*, **9**, 762—7 (1917).

Bottini, E. Pectin Materials in Fruits. *Industria chimica*, **5**, 285—7 (1930).

Brooks, R. O. Pectin Content of Certain Fruit Products. *Fruit Products J. and Am. Vinegar Ind.*, **4**, (7), 5—6 (1925); Citrus and Apple Pectins. *Ibid.*, **5** (4), 5—6 (1925).

Caldwell, J. S. A New Method for the Preparation of Pectin. Wash. Agr. Expt. Sta., *Bull.*, **147**, 3—14 (1917); Chemical Composition of the Juice of Some American Apples. *J. Agr. Research*, **36**, 407—17 (1928).

Campbell, C. H. Jelly. *Ind. Eng. Chem.*, **12**, 558—9 (1920).

Cameron, H., and Burns, R. B. Standardisation of Cherry and Blackberry Preserves and Blackberry Jelly. W. Va. Agr. Expt. Sta., *Bull.* **234**, 3—24 (1930).

Carré, M. H., and Haynes, D. Estimation of Pectin as Calcium Pectate and the Application of this Method to the Determination of the Soluble Pectin in Apples. *Biochem. J.*, **16**, 60—9 (1922); Changes which Occur in the Pectic Constituents of Stored Fruit. *Ibid.*, **16**, 704—12 (1922); Relation of Pectose and Pectin in Apple Tissue. *Ibid.*, **19**, 257—65 (1925); Investigations on the Pectic Constituents of Apples. *Ann. Botany.*, **39**, 811—39 (1925); An Investigation of the Behaviour of Pectic Materials in Apples and Other Plant Tissues. *Ibid.*, **41**, 193—237 (1927).

Charpentier, J. The Pectins obtained from Celery, the Tubercles of *Stachys tuberifera*, and the Peel of Bitter Orange. Application of the Biochemical Procedure for Characterisation of Galactose to the Study of the Composition of these Pectins. *Bull. soc. chim. biol.*, **6**, 142—56 (1924).

Codling, A. J., and Woodman, H. E. Sugar-Beet Pulp as a Source of Pectin. *J. Agr. Sci.*, **19**, 701—14 (1929).

Cole, G. M., Cox, R. E., and Joseph, G. H. Sugar Inversion and Other Phenomena in Jelly Making. *Food Manfr.*, **5**, 165—6 (1930).

Congdon, L. A. Investigation of Commercial Fruit Jellies. *North Dakota Special Bull.*, **3**, No. 8, 112—24 (1914).

Conrad, C. M. A Biochemical Study of the Insoluble Pectic Substances in Vegetables. *Am. J. Botany*, **13**, 531—47 (1926).

Cortese, D. The Hydrogen Ion Concentration of Pear Juices in Ripening. *Ann. chim. applicata.*, **20**, 197—204 (1930).

Cruess, W. V. Jellies and Marmalades from Citrus Fruits. Calif. Expt. Sta., *Circ.*, **146**, 1—8 (1916).

Dawson, J. A. The Chemical Composition of Loganberry Juice. *Can. Chem. Met.*, **10**, 81—4 (1926).

Daughters, M. R. Pectin and the Patents on its Manufacture. *Canning Age*, **7**, 110—2, 357—9 (1926).

Dore, W. H. Recent Progress in the Chemistry of Pectin and its Industrial Applications. *Ind. Eng. Chem.*, **16**, 1042—4 (1924); The Pectic Substances. *J. Chem. Education*, **3**, 505—13 (1926).

Eckart, H. Investigation of Commercial Apple Juices and Pectin Preparations. *Z. Untersuch. Lebensm.*, **50**, 405—15 (1925).

Eckart, H., and Gyalökay, A. V. *Konserven-Ind.*, **14**, 2—3, 13—5, 37—40 (1927).

Ehrlich, F., Constitution and Technical Importance of Pectins. *Chem.-Ztg.*, **41**, 197—200 (1917); Pectic Substances and their Significance in the Sugar Industry. *Deut. Zuckerind.*, **49**, 1045—50 (1924); Recent Investigations of Pectins. *Z. angew. Chem.*, **40**, 1305—13 (1927); Pectin Chemistry. *Cellulosechemie*, **11**, 140—51 (1930).

Ehrlich, F., and Sommerfeld, R. V. Composition of the Pectin Substances from Sugar Beets. *Biochem. Z.*, **168**, 263—323 (1626).

Ehrlich, F., and Schubert, F., Chemistry of the Pectin Substances: Tetra-galacturonic Acids and d-Galacturonic Acid from the Pectin of Sugar Beets. *Ber.*, **62B**, 1974—2027 (1929).

Ehrlich, F., and Kosmahly, A. Pectins of Fruits. *Biochem. Z.*, **212**, 162—239 (1929).

Emmett, A. M., and Carré, M. H. A Modification of the Calcium Pectate Method for the Estimination of Pectin. *Biochem. J.*, **20**, 6—12 (1926).

Emmett, A. M. A Comparison of the Various Methods of obtaining Ash-free Pectin. *Biochem. J.*, **20**, 564—8 (1926).

Farnell, R. G. W. Preliminary Investigations of the Pectic Substances of Plants. *Intern. Sugar J.*, **25**, 248—51 (1923); The Pectic Substances of Sugar Cane Fibre. *Ibid.*, **25**, 630—6 (1923); The Pectic and Pentosan Content of Cane Juices, Sirups and Molasses and its Removal. *Ibid.*, **26**, 480—6 (1924).

Fellenberg, T. v. The Pectins. *Mitt. Lebensm. Hyg.*, **5**, 225—6 (1914); Constitution of the Pectins. *Biochem. Z.*, **85**, 118—61 (1918).

Fellers, C. R. The Extraction of Apple Juices in the Manufacture of Jelly. Mass. Agr. Expt. Sta., *Res. Bull.*, **15**, 218—51 (1928).

Fellers, C. R., and Griffiths, F. P. Jelly Strength Measurement of Fruit Jellies by the Bloom Gelometer. *Ind. Eng. Chem.*, **20**, 857—9 (1928).

Fiedler, K. The Influence of the Sugar Inversion on the Gel Formation. *Konserven-Ind.*, **15**, 405—7, 427—8 (1928).

Gaddum, L. W. Study of Some of the Constituents of Citrus Fruits, Loquats, Roselle and Guava ; Pectin, Oils and Glucosides. Fla. Agr. Expt. Sta., *Ann. Rept.*, **1929**, 60—1.

Geret, L. Jelly Test for Jellying Fruit Juices and Pectin Preparations. *Mitt. Lebensm. Hyg.*, **21**, 116—7 (1930).

Graham, E. A., and Graham, H. T. Retardation by Sugars of Diffusion of Acids in Gels. *J. Am. Chem. Soc.*, **40**, 1900—17 (1918).

Griebel, C., and Northnagel, M. The Use of Pectin Preparations in the Manufacture of Marmalades, Jellies and Similar Fruits Products. *Z. Untersuch. Lebensm.*, **49**, 352—9 (1925).

Griebel, C., and Weiss, F. Pectin. *Ibid.*, **58**, 189—201 (1929).

Griggs. M. A., and Johnstin, R. Preparation and Colloidal Properties of Pectin. Preliminary Report. *Ind. Eng. Chem.*, **18**, 623—5 (1926).

Haller, M. H. Changes in the Pectic Constituents of Apples in Relation to Softening. *J. Agr. Research*, **39**, 739—46 (1929).

Halliday, E. G., and Bailey, G. R. Effect of Calcium Chloride on Acid-Sugar-Pectin Gels. *Ind. Eng. Chem.*, **16**, 595—7 (1924).

Hardy, F. Extraction of Pectin from the Fruit Rind of the Lime (*Citrus Medica Acida*). *Biochem. J.*, **18**, 283—90 (1924).

Haynes, D. Gelatinization of Pectin in Solutions of the Alkalies and the Alkaline Earths. *Biochem. J.*, **8**, 553—83 (1914).

Hirst, F. The Effect of Sugar, Acid and " Set " on the Keeping Properties of Jams. Univ. Bristol Agr. Hortic. Sta., *Ann. Rept.*, **1927**, 150—6.

Hornby, A. J. W. Pectins in Various Plants. *J. Soc. Chem. Ind.*, **39**, 246T (1920).

Huerre, R. Analysis of Lemon Juice. *J. pharm. chim.*, **20**, 5—9 (1919).

Hunt, C. H. Method for Preparing Pectin. *Science*, **48**, 201—2 (1918).

Jameson, E. Requirements of Pectin for the Commercial Jelly Maker. *Ind. Eng. Chem.*, **17**, 1291—2 (1925).

Johnstin, R., and Denton, M. C. The Relation of Alcohol Precipitate to Jellying Power of Citrus Pectin Extracts. *Ind. Eng. Chem.*, **15**, 778—80 (1923).

Jordan, S. Commercial Invert Sugar. *Ind. Eng. Chem.*, **16**, 307—10 (1924).

Joseph, G. H. Honey Jelly : Its Preparation and Properties. *Food Manfr.*, **5**, 257—8 (1930) ; A Note Concerning an Interesting Reaction between Pectin and Cows' Milk. *J. Soc. Chem. Ind.*, **49**, 159—160 T (1930).

King, J. The Adulteration of Conserves with Special Reference to Pectin and Agar-Agar. *Analyst*, **50**, 371—83 (1925).

Kopaczewski, W. The Coagulation of Pectin. *Bull. soc. chim. biol.*, **7**, 419—28 (1925).

Lathrop, C. A. Pure Fruit Jellies. *Canning Age* (Aug. 1925), 694—7.

Leo. H. T. The Proper Application of Pectin in Jelly Making. *The Canner*, **61** (2), 41—3 (1925).

Leone, P. Extraction of Pectins from Lemon Residues. *Ann. chim. applicata.*, **20**, 433—444 (1930).

Lindeman, J. Contribution to the Colloid Chemistry of Fruit Juices. A Study of the Conditions for the Making of Jelly from Apple Juice. *Meldinger Norges Landbruks.*, **7**, 157—297 (1927).

Lippold, E. "Obstkonserven, Fruchtsafte, Marmaladen." (Leipzig, 1916).

Lüers, H., and Lochmüller, K. Measuring the Jelly Strength of Fruit Pectins. *Kolloid-Z.*, **42**, 154—63 (1927).

Macara, T. The Composition of Fruits as Used for Jam Manufacture in Great Britain. *Analyst*, **56**, 35—43 (1931).

Magoon, C. A., and Caldwell, J. S. New and Improved Method for Obtaining Pectin from Fruits and Vegetables. *Science*, **47**, 592—4 (1918).

McGill, A. Jams. Lab. Inland Rev. Dept., Ottawa, Canada. *Bull.*, **302**, 23 pp. (1915).

McGuire, G., and Falk, F. G. Banana Gel. *J. Gen. Physiol.*, **4**, 437—45 (1922).

McKinney, J. W. Pectin. *J. Soc. Chem. Ind.*, **45**, 301—4T (1926).

Macmillan, A. M., and Tingle, A. Acidimetry of Red Wines and Fruit Juices. *Ind. Eng. Chem.*, **12**, 274—6 (1920).

McNair, J. B. Factors Determining the Consistency of Pectin Gels. *J. Phys. Chem.*, **20**, 633—9 (1916).

Mehlitz, A. The Influence of Acidity on the Formation of Pectin Compounds. *Z. tech. Biol.*, **11**, 134—54 (1925); Formation of Jellies. *Konserven-Ind.*, **12**, 467—70 (1925); Pectins. *Ibid.*, **13**, 1—3 (1926); Modification of Pectins during Cooking. *Chem. der Zelle u. Gewebe*, **12**, 353—61 (1926); Pectins. *Kolloid-Z.*, **41**, 130—46 (1927); Studies on Pectase. Part I. Enzymic Studies on Favourable Conditions for Pectase Coagulation. *Biochem. Z.*, **221**, 217—31 (1930).

Melis, B. Chemical Treatment of Lemon Residues. *Giorn. chim. ind. applicata.*, **11**, 399—404 (1929); Pectin from Lemons. *Ibid.*, **12**, 353—4 (1930).

Muttelet, C. F. The Acids of Fruits Used in Jam Making. *Ann. fals.*, **15**, 453—5 (1922).

Myers, P. B., and Baker, G. L. Fruit Jellies, Delaware Agr. Expt. Sta., *Bull.*, **141**, 14—9 (1925); Fruit Jellies; the *Rôle* of Salts. *Ibid.*, *Bull.*, **144**, 3—35 (1925); Fruit Jellies: the *Rôle* of Pectin: The Viscosity and Jellying Properties of Pectin Solutions.

Ibid., Bull., **149** (1927); Fruit Jellies: The *Rôle* of Pectin: The Extraction of Pectin from Pectic Materials. *Ibid., Bull.*, **160**, 1—64 (1929).

Nanji, D. R., Paton, F. J., and Ling, A. R. Decarboxylation of Polysaccharide Acids: its Application to the Establishment of the Constitution of Pectins and their Determination. *J. Soc. Chem. Ind.*, **44**, 253—8T (1925).

Nanji, D. R., and Norman, A. G. Studies on Pectin. II. The Estimation of the Individual Pectic Substances in Nature. *Biochem. J.*, **22**, 596—604 (1928).

Norman, A. G. The Biochemistry of Pectin. *Science Progress*, No. **94**, 263—79 (1929).

Norris, F. W., and Schryver, S. B. Pectic Substances of Plants: the Nature of Pectinogen and its Relation to Pectic Acid. *Biochem. J.*, **19**, 676—93 (1925).

Norris, F. W. The Pectic Substances of Plants: the Pectic Substances in the Juice of Oranges. *Ibid.*, **20**, 993—7 (1926).

Oden, S. Pectin Substances. *Intern. Z. phys.-chem. Biol.*, **3**, 71—82 (1917).

Ohn, A. The Viscosity of Pectin Sols. *Ind. Eng. Chem.*, **18**, 1295—8 (1926). The Viscosity of Pectin Sols: the Effects of Citric Acid and Various Sugars. *Ibid.*, **22**, 635—9 (1930).

Paure, H. S. Use of Pectin in Jams and Jellies. *Am. Food J.*, **17**, (3), 11—13 (1922).

Poore, H. D. Citrus Pectin. U.S. Dept. Agr., *Bull.*, **1323**, 1—19 (1925).

Papa-Ioannu, J. H. The Technology of the Pectins of Jellies and Marmalades. *Ann. Gembloux.*, **32** (3), 49—61; (4), 81—92; (7), 145—59; (8), 179—93 (1926).

Parrozani, M. Production of Pectin from the Waste Orange Peel and Pulp Remaining after the Oil Extraction. *Industrie chimique*, **14**, 117 (1927).

Paul, R. Apple Pectins. *Ann. fals.*, **22**, 78—82 (1929).

Paul, R., and Grandseigne, R. Extraction and Utilisation of Pectase. *Bull. assoc. chim. sucr. dist.*, **46**, 233—7, 245—6 (1929).

Perrier, S. Normal Presence of Starch in Pectic Juices Used in Jam Making. *Ann. fals.*, **17**, 208—11 (1924).

Poore, H. D. Effect of Dialysis on Direct Crystallisation of Citric Acid from Lemon Juice. *Ind. Eng. Chem.*, **15**, 775—8 (1923).

Romeo, G. Pectin from Bergamot. *Industria chimica*, **5**, 720—4 (1930). The Pectins. *Rivista ital. essenze e profumi.*, **12**, 24—7 (1930).

Romeo, G., and Sciacca, M. Pectin: Theory and Industry. *Giorn. chim. ind. applicata.*, **12**, 77—83 (1930).

Rooker, W. A. "Fruit Pectin." (New York, 1928); The Commercial Manufacture of Pectin Sirups and Powdered Pectins. *Fruit Products J. and Am. Vinegar Ind.*, **5** (1), 10—11, 18; (2), 10—12; (3), 10—12; (4), 10—12; (5), 14—16; (6), 10—13; (7), 11—14; (8), 11—12 (1925—26); New Uses of Fruit Pectin. *Ibid.*, **7** (1), 11—13 (1927).

Rosa, J. T. The Pectic Compounds of Water Melons and Citron Melons. *Am. J. Botany*, **14**, 631 (1927).

Savoja, G. Lemons as a Source of Pectin. *Industria chimica*, **4**, 367—9 (1929).

Sherwood, S. F. Jellying of Sorghum Sirup. *Ind. Eng. Chem.*, **15**, 780—2 (1923).

Singh, L. Relation of Pectin and Acidity in Jelly Making. *Ind. Eng. Chem.*, **14**, 710—11 (1922).

Smolenski, K. Pectins. *Roczniki Chemji*, **3**, 86—152 (1923).

Smolenski, K., and Wlostowska, W. Chemical Composition of Pectins. *Ibid.*, **7**, 591—611, 655—77 (1927); **10**, 328—41 (1930).

Spencer, G. Pectin Jellies. *Am. Colloid Symp. Monog.*, **4**, 302—3 (1926). The Formation of Pectin Jellies by Sugar. *J. Phys. Chem.*, **33**, 1987—2011 (1929); The Effect of Salts on Sugar-Pectin Jelly-Formation. *Ibid.*, **33**, 2012—20 (1929); The Relation between Acids and Pectin in Jelly Formation. *Ibid.*, **34**, 410—7 (1930); The Purification and Estimation of Pectin. *Ibid.*, **34**, 429—34 (1930); The Manipulation of Jelly-Strength Testing Apparatus. *Ibid.*, **34**, 654—5 (1930).

Sucharipa, R. Experimental Data on Pectin-Sugar-Acid Gels. *J. Assoc. Official Agr. Chem.*, **7**, 57—68 (1923); Protopectin and Some Other Constituents of Lemon Peel. *J. Am. Chem. Soc.*, **46**, 145—56 (1924). "Die Pektinstoffe" (Braunschweig, 1926).

Tarr, L. W. A Study of the Factors Affecting the Jellying of Fruits. Delaware Agr. Expt. Sta., *Bull.*, **133**, 14—15 (1923); Fruit Jellies: the *Rôle* of Acids. *Ibid., Bull.*, **134**, 1—37 (1923); Fruit Jellies. Bogue's "The Theory and Application of Colloidal Behaviour," Vol. 2, 605—26 (New York, 1924).

Tarr, L. W., and Baker, G. L. Fruit Jellies: the *Rôle* of Sugar. Delaware Agr. Expt. Sta., *Bull.*, **136**, 1—29 (1924).

Trier, C. Pectins. *Schweiz. Apoth. Ztg.*, **55**, 369—74 (1917).

Truelle, A. The Manufacture of Apple Jelly. *Vie agr. et rurale*, **6**, 357—61 (1916).

Tutin, F. Pectin and its Hypothetical Precursor "Propectin." *Biochem. J.*, **17**, 510—4 (1923).

Vercier, J. Studies on Strawberries. *Prog. agr. vit.*, **92**, 281—3 (1929).

Waele, H. de. Gelation of Pectin. *Ann. physiol. et physicochim. biol.*, **5**, 864—8 (1929).

Wendelmuth, G. The Gelation of Fruit Juices and Pectin. *Kolloidchem. Beihefte.*, **19**, 115—37 (1924).

Wichmann, H. J. Report on the Determination of Pectin in Fruit and Fruit Products. *J. Assoc. Official Agr. Chem.*, **6**, 34—40 (1922); **7**, 107—12 (1923); **8**, 123—30 (1924); Report on Pectin in Jams, Jellies and Preserves. *Ibid.*, **8**, 629—37 (1925).

Willeke, H., and Schellens, W. Statistics on Fruit Juices. *Z. Untersuch. Lebensm.*, **26**, 195—9 (1913).

Wilson, J. A. Retardation by Sugars of Diffusion of Acids in Gels. *J. Am. Chem. Soc.*, **41**, 358 (1919).

Wilson, C. P. Manufacture of Pectin. *Ind. Eng. Chem.*, **17**, 1065—7 (1925).

Ziegelmayer, W. Gelation of Fruit Juices. *Kolloid-Z.*, **52**, 243—8 (1930).

COLLOID PHENOMENA IN BREWING

Brown, H. T. On the Flocculation or "Break" of Worts and Beers and its Significance in Brewing Practice. *J. Inst. Brewing*, **19**, 84—121 (1913).

Brown, A. J. Colloid Chemistry in the Fermentation Industries. *Brit. Assocn. Coll. Repts.*, **2**, 57—60 (1918).

Chapman, A. C., and McHugo, C. W. Reaction of the Mashing Liquor in Relation to Extract Yield and Soluble Non-Coagulable Nitrogen. *J. Inst. Brewing*, **31**, 306—11 (1925).

Emslander, F. Surface Influences in the Diastase Process. *Kolloid-Z.*, **2**, 308—10 (1908); Water in the Brewery. *Ibid.*, **5**, 25—30 (1909); Further Contributions to the Knowledge of Colloid Chemical Aspects of Brewing. *Ibid.*, **6**, 156—64 (1910); Report on Recent Advances in the Colloid Chemistry Relating to Brewing Industry. *Ibid.*, **7**, 177—82 (1910); Colloid Chemistry of Beer in 1911. *Ibid.*, **10**, 189—96 (1910); Colloid Chemistry and Brewing. Liesegang's "Kolloidchemische Technologie" (Dresden, 1931).

Freundlich, H. The Importance of Colloid Chemistry to the Fermentation Industry. *Z. angew. Chem.*, **35**, 517—9 (1922).

Grieg-Smith, R. The Influence of Certain Colloids upon Fermentation. *Proc. Linnean Soc. N.S. Wales*, **49**, 436—46 (1924).

Hatschek, E. Colloidal Chemistry and Brewing. *J. Inst. Brewing*, **18**, 494—522 (1912).

Heintz, L. Artificial Cooling of Worts in Closed Coolers and the Elimination of Glutin. *Wochschr. Brau.*, **34**, 221—4, 230—5 (1917).

Hopkins, R. H., and Burns, J. A. Proteolytic Enzymes of Green Malt. *J. Inst. Brewing*, **36**, 9—15 (1930).

Horsch, R., and Schulteis. The Boiling of Wort and Fermentation. *Wochschr. Brau.*, **46**, 295—8 (1929).

Huyghe, A., and Eynde, G. V. Den. Hops. *Bull. assocn. élèves inst. sup. fermentations Gand.*, **28**, 348—65 (1927).

Jalowetz, E. Boiling of Brewery Wort. *Wochschr. Brau.*, **46**, 483—6 (1929); Technology of Malting: (1) Cold and Warm Flooring Method. *Brau.-Malzindustrie*, **1**, 1—5 (1930); (2) Influence of the Composition of the Steep Water. *Ibid.*, **2**, 25—7 (1930).

Lintner, C. J. The Colloid Condition of Brewing Materials and its Importance in Brewing. *Z. ges. Brauw.*, **32**, 633—8 (1909).

Lüers, H., and Baumann, A. The Analysis of Hop Bitter Acids and Their Changes during the Hop Boiling. *Z. ges. Brauw.*, **43**, 65—7, 73—6, 81—4, 89—92, 97—101 (1920); Colloid Chemical Studies on the Bitter Acids of Hops. *Kolloid-Z.*, **26**, 202—12 (1920).

Marc, R. Estimation of Colloids in Beers. *Kolloid-Z.*, **14**, 181—6 (1914).

Moritz, F. E. B., and Fuller, D. H. F. Modification of Malt in Relation to the Stability of Beer. *J, Inst. Brewing*, **32**, 269—78 (1926).

Nakamura, Y. Chemical Studies on the Brewing Barley. *J. Fac. Agr. Hokkaido Imp. Univ.*, **23**, 29—86 (1928).

Ranken, C. Surface of Yeast as a Factor in Fermentation. *J. Inst. Brewing*, **33**, 76—84 (1927).

Rechenberg. Mash-boiling under Pressure (the Lazarus Process) Results in Beers of Exceptional Body and Head-Forming Power. *Z. ges. Brauw.*, **44**, 184—6 (1921).

Reichard, A. Studies on Beer Colloids. *Z. ges. Brauw.*, **34**, 253, 265 (1911).

Remy, E. Recent Investigations on the Chemical and Physical Composition of Light Lager. *Z. Untersuch. Lebensm.*, **59**, 402—6 (1930).

Schoellhorn, F. "Bibliographie des Brauwesens" (Berlin, 1928).

Schryver, S. B. The Chemistry of Proteins and its Importance in Brewing. *J. Inst. Brewing*, **35**, 532—8 (1929).

Smith, L. G. Notes on the Nitrogen Content of Worts and their Beers. *J. Inst. Brewing*, **32**, 220—5 (1926).

Söhngen, H. L. Influence of Some Colloids on Alcoholic Fermentation. *Folia Microbiologica*, **2**, 94 (1913).

Windisch, W., and Dietrich, W. Changes in the Titratable Acidity, Surface Tension and Colour of Wort and Fermented Wort on Fractional Ultrafiltration. *Biochem. Z.*, **105**, 96—110 (1920); *Wochschr. Brau.*, **37**, 231—3, 237—9 (1920).

Windisch, W. (with Kolbach, Wentzell, Borges). Conversion of α-Hop Bitter Acid during Boiling in Aqueous Solution. *Wochschr. Brau.*, **41**, 281—3, 289—92, 299—302 (1924) Changes in the Degree of Dispersion of the Proteins of Barley during Malting, Mashing and Fermentation. *Ibid.*, **42**, 287—8, 295—7, 303—6, 313—7, 323—5, 333—5 (1925); Protein Coagulation during the Boiling of Unhopped Wort. *Ibid.*, **46**, 403—6, 417—9 (1929); Electrodialysis of Wort and Beer. *Ibid.*, **47**, 67—71, 77—81 (1930).

pH in BREWING

Bermann, P. The Significance of Buffer Substances in the Fermentation Industries. *Wochschr. Brau.*, **42**, 267—8, 273—5 (1925).

Britton, H. T. S. The Importance of Hydrogen Ion Concentration in Brewing. *Ind. Chem.*, **4**, 150—3 (1928).

Emslander, F. Concentration of Hydrogen Ions in Beer. *Kolloid-Z.*, **13**, 165—70 (1913); **14**, 44—8 (1914); Concentration of Hydrogen Ions in Beer and its Relation to the Brewing Process. *Z. ges. Brauw.*, **37**, 2 (1914); *Wochschr. Brau.*, **33**, 170—1 (1916); Concentration of Hydrogen Ions in Beer. *Z. ges. Brauw.*, **42**, 127—30, 135—7 (1919).

Gronckel, C. de. The pH Value of Beer. *Bull. assocn. élèves inst. sup. fermentations Gand.*, **31**, 289—300 (1930).

Hagues, G. Hydrogen Ions in Brewing Processes. *J. Inst. Brewing*, 32, 8—19 (1926) ; **33**, 262—76 (1927) ; **34**, 362—76 (1928) ; **35**, 51—60 (1929).

Heintz, L. Titration in Stages. *Wochschr. Brau.*, **46**, 393—6 (1929).

Hopkins, R. H. Contributions to Our Knowledge of Hydrogen Ions in Brewing. *J. Inst. Brewing*, **31**, 399—413 (1925).

Hopkins, R. H., and Kelly, H. E. The Formation of Buffer Substances in Malting. *Ibid.*, **35**, 402—9 (1929).

Kolbach, P. Buffer Substances in Wort and Beer. *Wochschr. Brau.*, **43**, 123—9, 135—40, 277—80, 289—94 (1926) ; Significance of Acidity in the Preparation of Beer. *Ibid.*, **47**, 145—9, 163—8 (1930).

Laer, M. H. V. The Function of pH in Brewing. *Chimie et industrie*, **14**, 511—8 (1925).

Leberle, H., and Lüers, H. Acid Determination in Beer by Electrochemical Methods. *Z. ges. Brauw.*, **37**, 177—84 (1914).

Lüers, H. The Significance of Hydrogen Ion Concentration in the Brewery. *Wochschr. Brau.*, **44**, 97—103 (1927).

Monfang, E. Acidity and Stability of Beer. *Kolloid-Z.*, **5**, 275 (1909).

Parsons, N. M. Electric Measurement of Available Acidity and its Applications in English Brewing. *J. Inst. Brewing*, **30**, 30—56 (1924).

Petit, P. Determination and Function of pH. *Brasserie malterie*, **17**, 241—6 (1927).

Windisch, W., and Dietrich, H. Titrations with Capillary-Active Substances as Indicators : Buffer Substances in Wort and Beer. *Wochschr. Brau.*, **37**, 255—7, 261—4 (1920).

Windisch, W., and Kolbach, P. Influence of the Mashing Process and pH on the Composition of the Wort and the Acidity of the Beer. *Ibid.*, **44**, 237—40, 243—6 (1924) ; Influence of the Brewing Water on the Acidity of the Wort and Beer. *Ibid.*, **43**, 423—8, 444—7 (1926) ; Influence of Hydrogen Ion Concentration on Development of Colour during Wort Boiling. *Ibid.*, **44**, 53—6, 64—7 (1927),

HEAD-FORMATION AND FOAMING

Baker, J. L., and Hulton, H. F. E. Head Retention in Beer. *J. Inst. Brewing*, **29**, 747—75 (1923).

Bartsch, O. Foam Systems. *Kolloidchem. Beihefte*, **20**, 1—50 (1924) ; Foaming Power and Surface Tension. *Kolloid-Z.*, **38**, 177—8 (1926).

Clayton, W. Colloid Chemistry of Technical Froths and Foams. *Ind. Chem.*, **1**, 489—91 (1925).

Clerck, J. de. Surface Tension and the Part it Plays in Brewing. *Bull. assocn. étudiants école sup. brasserie Univ. Louvain*,. **30**, 127—36 (1930) ; Measuring the Surface Tension of Beer and Worts. *Ibid.*, **30**, 136—50 (1930).

Creighton, H. J. M. The Influence of Colloids on the Solubility of Gases. Alexander's " Colloid Chemistry " (New York, 1926), Vol. 1, 634—6.

Emslander, F., and Freundlich, H. Surface Influences in Relation to Beer and Brewing. *Z. physik. Chem.*, **49**, 317—28 (1904).

Emslander, F. Foam Stability : the Danysz Phenomenon. *Wochschr. Brau.*, **38**, 418—23 (1926) ; Fermentation and Vat Walls. *Ibid.*, **46**, 401—3 (1929).

Fernbach, A. Head on Beer. *Ann. Brass. Dist.*, **16**, 145 (1913).

Foulk, G. W. A Theory of Liquid Film Formation. *Ind. Eng. Chem.*, **21**, 815—7 (1929).

Fürnrohr, O. Foam-Holding Capacity of Beer. *Z. ges. Brauw.*, **36**, 473—5, 481—8 (1914).

Gans, D. M., and Harkins, W. D. The Direct Measurement of the Adsorption of Soluble Substances by the Bubble Method. *J. Phys. Chem.*, **35**, 722—39 (1931).

Geys, K. Measurement of Foam in Beers and the Factors Bearing on This. *Wochschr. Brau.*, **43**, 439—44 (1926) ; Head on Beer from the Physico-Chemical Standpoint. *Ibid.*, **44**, 145—7 (1927).

Horn, D. W. Some Anomalous Tensions. *Am. J. Pharm.*, **98**, 53—8 (1926).

Johlin, J. M. Interfacial Tension as a Function of the Concentration of Colloidal Solutions. *J. Biol. Chem.*, **84**, 543—51 (1929).

Kalk. Effect of the Method of Conducting Fermentation on the Stability of the Foam, CO_2 Content and Stability of Beers. *Brasserie malterie*, **15**, 117—23 (1925) ; Determination of the CO_2 Content of Beer and the Stability of the Foam. *Ibid.*, **15**, 123—6 (1925).

King, G. The Solubility, Rate of Absorption and Evolution of Gases as Influenced by Colloids, With Special Reference to Physiology and Brewing. *Brit. Assocn. Coll. Repts.*, **3**, 41—7 (1920).

King, J. Surface Tension of Liquids Met with in Biochemistry with Special Reference to Brewery Worts. *J. Inst. Brewing*, **31**, 32—7 (1925).

Lüers, H., Geys, K., and Baumann, A. Beer Foam. *Z. ges. Brauw.*, **43**, 185—7, 193—5, 201—3 (1920).

Lüers, H., and Schmal. Study of the Foaming Qualities of Beers. *Brasserie malterie*, **16**, 90—4 (1926).

Murray, H. D. Rapid Determination of the Gases Dissolved in Beer and Other Colloidal Liquids. *J. Inst. Brewing*, **31**, 137—9 (1925).

Ostwald, Wo., and Steiner, A. Foaming-Power and Surface Tension Particularly with Humus Sols. *Kolloid-Z.*, **36**, 342—51 (1925).

Ostwald, Wo., and Meissner, M. Studies on Membrane Effects with Colloidal Solutions. *Kolloidchem. Beihefte*, **26**, 1—57 (1928).

Peard, G. T., and Johnston, J. H. St. Surface Tension of Wort and Beers. *J. Inst. Brewing*, **31**, 416—25 (1925).

Petit, P. Mellowness and Foam. *Brasserie malterie*, **16**, 49—53, 65—8, 81—4 (1926) ; Colloids and the Stability of Beer. *Ibid.*, **20**, 161—4, 177—80 (1930).

Ralk, C. Frothing Test as a Criterion of the CO_2 Content and Head Retention of Beer. *Wochschr. Brau.*, **42**, 76—7 (1925) ; Influence of Mode of Fermentation on the Head-Retention, CO_2 Content and Stability of Beer. *Ibid.*, **42**, 79—81, 85—7 (1925).

Ramsden, W. Surface Films. *Trans. Liv. Biol. Soc.*, **33**, 1—24 (1919); Some Physical Properties of Composite Surfaces. *Trans. Faraday Soc.*, **22**, 484—5 (1926).

Raux, J. Modification of the Head of Beer by Iron. *Brasserie malterie*, **15**, 181—4 (1925).

Rehbinder, P., and Wenström, E. Stabilising Action of Adsorbed Layers of Surface-Active Substances on Disperse Systems: Stability of Bubbles and Drops in Boundary Surfaces. *Kolloid-Z.*, **53**, 145—58 (1930).

Rigaux, R. Froth on Beer. *Ann. Inst. sup. fermentations Gand.*, **34**, 93—106 (1925).

Schmidt, H. The Effect of Shaking on Serum, *J. Hyg.*, **14**, 399—415 (1914).

Schnurmann, R. The Size of Gas Bubbles in Liquids. *Z. physik. Chem.*, **Abt. A, 143**, 456—74 (1929).

Stark, H. M. The Coagulation of Ferric Oxide Sols by Gas Bubbles. *J. Am. Chem. Soc.*, **52**, 2730—42 (1930).

Williams, H. E. Foam Meter. *Ind. Eng. Chem.*, **18**, 361—2 (1926).

Wilson, R. E., and Ries, E. D. Surface Films as Plastic Solids. *Am. Coll. Symp. Monog.*, **1**, 145—73 (1923).

Windisch, W., and Bermann, V. Conditions Necessary for Head Formation by Beer Wort. *Wochschr. Brau.*, **37**, 109—11, 121—5, 129—32, 137—9, 145—7, 153—5 (1920).

Windisch, W., Kolbach, P., and Banholzer, W. Determination of the Head Forming and Head Retention Powers of Beer. *Ibid.*, **42**, 239—40, 251—3 (1925); Influence of Hop Constituents on Head Formation in Beer. *Ibid.*, **43**, 207—9, 217—23, 229—35, 241—6 253—8 (1926).

SAPONINS

Bonnet, A. P. Saponins. *Parfum moderne*, **17**, 118—9 (1924).

Dafert, O. Saponins. *Oesterr. Chem.-Ztg.*, **30**, 6 (1927).

Karsmark, K. A. Saponins and Saponin Drugs. *Svensk Farm. Tids.*, **29**, 453—9, 467—72 (1925).

Kofler, L. Surface Activity and the Toxicity of the Saponins. *Biochem. Z.*, **129**, 64—72 (1922); Differentiation and Quantitative Estimation of the Saponins. *Z. Untersuch. Lebensm.*, **43**, 278—87 (1922); The Valuation of Saponin as a Foam-Forming Material. *Chem.-Ztg.*, **48**, 165—6 (1924).

Kofler, L., and Wolkenberg, A. The Behaviour of Saponin during Dialysis. *Biochem. Z.*, **160**, 398—406 (1925).

Kofler, L. Chemical, Physical and Biological Properties of the Saponins. *Protoplasma*, **7**, 106—28 (1929).

Kroeber, L. Saponins. *Apoth. Ztg.*, **43**, 371—3 (1928).

Mand, R. The Valuation of Saponin as Foam Producer. *Chem.-Ztg.*, **50**, 850—1 (1926).

Poethke, W. Saponins and Their Importance in Pharmacy. *Pharm. Zentralhalle*, **71**, 753—61 (1930).

Wasicky, R. The Saponins. *Wiener klin. Wochschr.*, **39**, 1095—7 (1926).

Wastl. H. The Surface Tension of Saponin Solutions, *Biochem. Z.*, **146,** 376—9 (1924).

Woodward, H. E., and Alsberg, C. L. Comparison of the Effect of Certain Saponins on the Surface Tension of Water with Their Hemolytic Power. *J. Pharmacol.*, **16,** 237—45 (1920).

HAZE IN BEER

Ash, C. S. Ash Process of Treating Beer. *Western Brewer*, **50,** 93—4 (1918).

Bekaert, E. Turbidity of Beer due to Amorphous Nitrogen Compounds. *Chimie et industrie*, **19,** 415 (1928).

Cauwenberge, P. V. The Effect of pH Value on the Clarification of Beers. *Chimie et industrie*, **19,** 415 (1928).

Delroisse, A. Cause of Haze in Beer Due to Caramel. *Petit J. Brass*, **32,** 636 (1924).

Disez, A. Influence of the Bottle Glass on Stability of Beer *Wochschr. Brau.*, **40,** 115—9 (1923).

Emslander, F. Ultrafiltration in the Brewery. *Kolloid Z.*, **2,** 88 (1907). Cause of Precipitation in Finished Pasteurised Bottled Beer. *J. Inst. Brewing*, **18,** 484 (1912) ; What is the Cause of Protein Haze in Bottled Beer, although Normally Handled in the Brewing Room and the Store. *Kolloidchem. Beihefte*, **3,** 47—84 (1912) ; Colloids as Vehicles of Undesirable Flavours in Beer. *Wochschr. Brau.*, **30,** 387 (1913).

Fink, H., and Wildner, H. Tannin : Precipitation of the Proteins of Wort and Beer by Tannin. *Wochschr. Brau.*, **47,** 11—16, 23—6 (1930).

Fink, H., and Riedel, W. Determination of the " Tannin-Nitrogen " and the " Tannin-Number " as a New Method for Defining the Protein Conditions in Beer. *Ibid.*, **47,** 491—5, 501—5, 511—15 (1930).

Furnrohr, O. The Sensitivity of Beer to Chilling. *Z. ges. Brauw.*, **32,** 589 (1909) ; Changes in the Constitution of Proteins in Beer Due to Heat and Cold. *Ibid.*, **36,** 429, 438 (1914).

Geys, K. Oxalic Acid Turbidity and Related Problems. *Z. ges. Brauw.*, **45,** 2—5, 9—11 (1922).

Gronckel, de. The Pasteurisation of Beer. *Bull. assocn. élèves inst. sup. fermentations Gand.*, **29,** 335—41 (1928).

Harman, H. W., and Oliver, J. H. Caramel and Stability (of Beer). *J. Inst. Brewing*, **31,** 577—80 (1925).

Harman, H. W., Oliver, J. H., and Woodhouse, P. Finings. *Ibid.*, **34,** 203—13 (1928).

Hartong, B. D. Separation of Protein-Tannin during the Boiling of Wort. *Wochschr. Brau.*, **46,** 543—5 (1929) ; **47,** 295—7 (1930).

Hesselberger, W. Protein of Different Barleys and Turbidities Produced by Pasteurisation. *Wochschr. Brau.*, **46,** 285—8 (1929).

Hind, H. L. Haze in Bottled Beer. *J. Inst. Brewing*, **37,** 15—19 (1931).

Hopkins, R. H., and Fraser, J. E. The Biological Stability of Beer. *J. Inst. Brewing*, **34,** 387—402 (1928).

Lüers, H. Pasteurisation of Beer and Turbidities Caused Thereby. *Z. ges. Brauw.*, **45,** 159—62, 164—8 (1922) ; Clarification during the Brewing Operations. *Ibid.*, **53,** 55—8 (1930).

Netscher H. Silicic Acid in Beer. *Wochschr. Brau.*, **45,** 582—5 (1928), **46,** 335—7 (1929).

Petit, P. Sensitiveness of Beer Towards Cold and the Wallerstein Process. *Brasserie malterie*, **6,** 161—6 (1916).

Petit, P., and Raux, J. Clarification of Beer by Super-Centrifuging. *Ibid.*, **16,** 276—83 (1926).

Petit, P. Observations on Beers and Their Stability. *Ibid.*, **19,** 241—7 (1929) ; Turbidity on Pasteurising Beer. *Ibid.*, **19,** 353—7 (1930) ; Yeast Turbidity of Beer. *Ibid.*, **20,** 209—12, 225—8 (1930).

Ranken, C. The Use of Irish Moss as Copper Finings. *J. Inst. Brewing*, **35,** 287—91 (1929).

Raux, J. The Manufacture of High-Grade Beers. *Brasserie malterie*, **19,** 279—85 (1929).

Reichard, A. Reactions of Tannin and Their Importance in Brewing. *Kolloid-Z.*, **10,** 209—14 (1912).

Schonfield, F., and Hirt, W. Metal-Protein Turbidity of Pale Beers. *Wochschr. Brau.*, **27,** 633 (1910) ; **29,** 557 (1912).

Stadler, H. Effect of the Protein Content of Barley on Quality of Dark Beer. *Wochschr. Brau.*, **46,** 479—83 (1929).

Stockhausen, F. Electric Charges on Yeast and Hydrogen Ion Concentration and Their Influence on Attenuation and Flocculation. *Wochschr. Brau.*, **44,** 121—4, 133—8 (1927).

Vanlehrberghe, J. The Isoelectric Point for the Clarification of Beer. *Bull. assocn. élèves inst. sup. fermentations Gand.*, **28,** 332—41 (1927).

Verbrugghen, W. The Pasteurisation of Beers. *Ibid.*, **30,** 354—61 (1929).

Visez, A. Haze in Export Beer. *Wochschr. Brau.*, **39,** 33—5, 38—9 (1922) ; Manufacture of Protein-Stable Beer and the Relation of Dextrin Content to Protein Stability. *Ibid.*, **39,** 73—5 (1922) ; Influence of the Composition of Bottle Glass on the Stability of Beer. *Bull. école sup. brasserie Louvain*, **23,** 68—84 (1923).

Vossen, A. Lambic Beers. *Bull. assocn. élèves inst. sup. fermentations Gand.*, **31,** 159—64 (1930).

Wallerstein, L. Enzymes in the Fermentation Industries. *J. Franklin Inst.*, Nos. 5 and 6 (1917).

Will, H. Calcium Fluoride as Cause of Beer Haze. *Z. ges. Brauw.*, **37,** 357—8 (1914) ; Influence of Shaking on the Biological Stability of Beer. *Ibid.*, **40,** 249—52 (1917).

Windisch, W. Pre-Mashing, Proteolytic Digestion and Protein Turbidity. *Wochschr. Brau.*, **33,** 105—8, 121—5 (1916) ; Haze and Haze Flavour. *Ibid.*, **46,** 325 (1929).

WINES

Astruc. H. Centrifuging Wines. *Prog. agr. vit.*, **92,** 60—4 (1929).

Baragiola, W. T. The White Turbidity of Wines. *Z. Untersuch. Lebensm.*, **33,** 513—20 (1917).

Bosselmann, H. The Clarification of Wines with Potassium Ferrocyanide. *Z. Untersuch. Lebensm.*, **47**, 209—14 (1924).

Brown, H. T. White Wines of South Africa. *J. Inst. Brewing*, **20**, 345—75 (1914).

Casale, L. The Determination of the Concentration of Hydrogen Ions in Wines. *Staz. sper. agrar. ital.*, **52**, 375—88 (1919) ; Physico-Chemical Investigations of Wines. *Ibid.*, **59**, 143—206 (1926).

Castella, F. de. Modern Wine-Making Methods. *J. Dept. Agr. Victoria*, **27**, 651—60 (1929).

Dubaquié, J. Effects of SO_2 on the Behaviour of Bottled White Wines. *Ann. fals.*, **18**, 418—22 (1925) ; **19**, 459—62 (1926) ; Tests on the Clarification of Liqueur Wines by centrifuging. *Ibid.*, **21**, 460—3 (1928).

Errichelli, E. The Influence of Potassium-Hydrogen Tartrate, Tartaric Acid and Malic Acid on the Flocculation of Proteins in Relation to the Defecation and Clarification of Musts. *Ann. chim. applicata*, **17**, 221—44 (1927).

Farbre, J. H., and Brèmond, E. Total Iron Content of Wines. *Ann. fals.*, **20**, 213—7 (1927).

Fonzes-Diacon. The White Casse of Wines. *Compt. rend.*, **164**, 199—200, 650—2 (1917).

Heide, C. V. D. The Influence of Clarifying on the Chemical Constituents of Wine. *Landw. Jahrb.*, **45**, *Ergänzbd.*, **1**, 134—8 (1914).

Hewitt, J. T. The Chemistry of Wine Making, a Report on Œnological Research. (London, H.M. Stationery Office, 1928.)

Krug. The Clarification of Wine with Potassium Ferrocyanide. *Z. Untersuch. Lebensm.*, **48**, 96—103 (1924).

Laborde, J. White Casse of Wines. *Compt. rend.*, **164**, 441—3 (1917).

Levallois, F. Tannins in the Wine Industry. *Ann. fals.*, **19**, 15—28 (1926).

Malvezin, P., and Essner, J. C. Characterisation and Estimation of the Iron Casse in Wines. *Ann. fals.*, **17**, 473—7 (1924).

Malvezin, P. Application of Adsorption to Sterilisation of Wines. *Bull. soc. chim.*, **47**, 1194—6 (1930).

Manceau, E. Scientific Control in the Preparation of Bottled Champagne. *Ann. fals.*, **20**, 7—19 (1927).

Marca, F. L. Influence of Clarification on the Composition of Wines. *Staz. sper. agrar. ital.*, **48**, 185—232 (1915).

Marsais, P. The " Blue " of Champagne Wines. *Rev. vit.*, **72**, 283—7 (1930).

Mathieu, L. Theory and Practice of the Clarification of Wines. *Chimie et industrie*, Special No., 608—14 (Sept., 1925).

Mayr, E., and Rüdiger, M. The Clearing of Wines. *Kolloid-Z.*, **47**, 141—54 (1929).

Morani V., and Marimpietri, L. Buffering Power of Wines : Keeping and Plastering. *Ann. chim. applicata*, **20**, 313—35 (1930).

Moreau, L., and Vinet, E. Studies in the Vinification of Chemin Grapes : Use of Tannin. *Rev. vit.*, **41**, 457—61 (1914) ; Addition of

Ammonium Phosphate. *Ibid.*, **41**, 679—82 (1914); Study and Treatment of White Casse in Wines. *Bull. soc. agr. France*, (**1917**), 267—71, 292—9 (1919), 16—7.

Muller-Thurgau, H., and Osterwalder, A. The Browning of Wines. *Landw. Jahrb. Schweiz.*, **37**, 215—39, 241—74 (1923).

Piedallu, A. The *Rôle* of Iron in the Blue Casse of Wine. *Compt. rend.*, **169**, 1108—9 (1919); **170**, 1129—31 (1920).

Priore, G. L. The Milk Turbidity of White Wines. *Staz. sper. agrar. ital.*, **50**, 382—90, 394 (1917).

Reichard, O. Clarification of Wine. *Z. Untersuch. Lebensm.*, **53**, 163—8 (1927); Clarification with Potassium Ferrocyanide. *Ibid.*, **58**, 488—505 (1929).

Rüdiger, M., and Mayr, E. The Clarification of Wines. *Z. angew. Chem.*, **41**, 809—12 (1928); *Kolloid-Z.*, **46**, 81—9 (1928).

Semichon, L. Grape Pectins and the Mellowness of Wines. *Chimie et industrie*, **17**, 25—32 (1927).

Semichon, L., and Flanzy, M. Grape Pectins and the Mellowness of Wines. *Compt. rend.*, **183**, 394—6 (1926); Constitution and Estimation of Pectins and Gums in Grape Wines and Musts. *Ann. fals.*, **20**, 395—9 (1927).

Taüfel, K., and Wagner, C. Significance and Value of the Titration Curves of Wine. *Z. anal. Chem.*, **71**, 1—23 (1927).

Weil, H. The Clouding of Wines Through Iron Phosphate Compounds. *Z. Untersuch. Lebensm.*, **29**, 60—6 (1915).

Widmer, A. Methods of Restoring Turbid Grape and Fruit Wines. *Schweiz. Apoth. Ztg.*, **57**, 627—32, 639—45, 651—7 (1919).

Wolff, J., and Grandchamp, L. Oxidisability of Iron contained in Certain Wines. *Compt. rend.*, **181**, 939—41 (1925).

COLLOID PROBLEMS IN FREEZING

Acklin, O. Researches on the Keeping Qualities of Frozen Flesh with Special Reference to its Use in Sausage. *Z. Untersuch. Lebensm.*, **55**, 31—44 (1928).

Aggradi, M. Freezing Point of Pears and Apples. *Ann. chim. applicata.*, **20**, 188—96 (1930).

Almy, L. H., and Field, E. The Preservation of Fish Frozen in Chilled Brine: (1) The Penetration of Salt. *Ind. Eng. Chem.*, **13**, 927—30 (1921); (2) The Keeping Quality of the Fish. *Ibid.*, **14**, 203—6 (1922).

Anon. The Ottesen Method of Preserving Fish. *Boll. Pesca, Piscicolt. e. Idrobiol.*, **3** (2), 3—14 (1927).

Anon. The Differentiation of Thawed Frozen Meat from Fresh Meat. *Mitt. Lebensm. Hyg.*, **19**, 219—22 (1928).

Bailey, C. H., and Bayfield, E. G. Water Imbibition of Frosted Wheat. *Cereal Chem.*, **7**, 108—116 (1930).

Bailey, H. S., and Wilson, C. P. The Composition of Sound and Frozen Lemons with Special Reference to the Effect of Slow Thawing on Frozen Lemons. *Ind. Eng. Chem.*, **8**, 902—4 (1916).

Barnicoat, C. R. Freezing of Bacon. *N. Zealand J. Sci. Tech.*, 11, 409—10 (1930).

Barrier, A., and Monwoisin, A. On the Technique of Thawing Meat. *Rev. gén. Froid.*, **9**, 83 (1928).

Barry, J. J., and Herter, C. H. American Improvements in Freezing Meat and Fish. *Proc. 4th Intern. Cong. Refrig.* (*London*), **2**, 1079—91 (1924).

Bělehrádek, J. Retardation of Biological Reactions by Cold is Caused by an Increase in the Viscosity of Protoplasm. *Protoplasma*, **3**, 317 (1928).

Bergami, G. Action of Low Temperatures on the Crystalline Lens. *Atti. accad. Lincei.*, **6**, 117—21 (1927).

Birdseye, C. Some Scientific Aspects of Packaging and Quick-Freezing Perishable Flesh Products : More Rapid Freezing Means Better Preservation. *Ind. Eng. Chem.*, **21**, 414—7 (1929).

Bottazzi, F., and Bergami, G. Action of Low Temperatures on Fluid Colloidal Systems. *Arch. sci. biol. Ital.*, **6**, 74—93 (1924).

Bottazzi, F. Action of Temperature on Tissues and Their Colloidal Components : Cold Rigidity. *Atti. accad. Lincei.*, **1**, 68—70 (1925).

Bottemanne, J. M., and Jong, D. A. de. A Report on the Freezing of Fish. *Proc. 4th Intern. Cong. Refrg.* (*London*), **2**, 1211—17 (1924).

Brooks, J. The Effect of Freezing in a Concentrated Solution of NaCl on the Colour of Red Muscle. *Biochem. J.*, **24**, 1379—83 (1930).

Callow, E. H. Autolysis of the Muscle of the Codfish. *Ibid.*, **19**, 1—6 (1925) ; Velocity of Ice Crystallisation Through Supercooled Gelatin Gels. *Proc. Roy. Soc.*, **A 108**, 307—23 (1925).

Caro, L. de. The Production of Lactic Acid and of Phosphoric Acid in the Rigidity of Thawing. *Atti. accad. Lincei.*, **6**, 607—12 (1927).

Carrick, D. B. Cold Storage and Freezing Studies on the Fruit of the Vinifera Grape. Cornell Univ. Agr. Expt. Sta., *Mem.* **131**, 3—37 (1930).

Clark, E. D., and Pennington, M. E. The Commercial Freezing and Storing of Fish. U.S. Dept. Agr. *Bull.*, **635**, 1—9 (1918).

Clark, E. D., and Almy, L. H. A Chemical Study of Frozen Fish in Storage for Short and Long Periods. *Ind. Eng. Chem.*, **12**, 656—63 (1920).

Clifford, W. M. Effect of Short Periods of Cold Storage on Beef and Mutton. *Biochem. J.*, **19**, 998—1003 (1925).

Colella, C. The Aseptic Autolysis of Frozen Meat. *Boll. soc. ital. biol. sper.*, **3**, 320—4 (1928).

Cook, G. A., *et al.* Refrigeration of Meat : I. Beef. *Australian J. Exp. Biol. Med.*, **3**, 15—31 (1926).

Corblin, B. The Cooling and Freezing of Milk. *Bull. soc. encourage ind. nat.*, **1928**, 232—42.

Crane, H. L. Physiological Investigations on the Resistance of Peach Buds to Freezing Temperatures. W. Va. Agr. Expt. Sta., *Bull.*, **236**, 5—80 (1930).

Dhéré, C. D. Action of Freezing on Certain Physiological Colloids. *Arch. sci. biol.* (*Italy*), **7**, 278—85 (1925).

Emmett, A. D., and Grindley, H. S. A Preliminary Study of the Effect of Cold Storage upon Beef and Poultry. *Ind. Eng. Chem.*, **1**, 580 (1909).

Fearon, F. R., and Foster, D. L. Autolysis of Beef and Mutton. *Biochem. J.*, **16**, 564—71 (1922).

Foote, H. W., and Saxton, B. Effect of Freezing on Certain Inorganic Hydrogels. *J, Am Chem. Soc.*, **38**, 588—609 (1916).

Foster, D. L., and Moyle, D. M. Effects of Exposure to Low Temperatures on Some Physiological, Chemical and Physical Properties of Amphibian Muscle. *Biochem. J.*, **15**, 334—46 (1921).

Foster, D. L. Some Problems of the Freezing of Beef Presented to a Biochemist. *Proc. 4th Intern. Cong. Refrig.* (*London*), **1**, 247—50 (1924).

Gonzalez, L. G. Some Freezing Studies on Celery. *Proc. Am. Soc. Horticult. Sci.*, **23**, 339 (1926).

Hardy, W. B. Scientific Problems of Cold Storage Industries. *J. Am. Soc. Refrig. Eng.*, **6**, 201—8 (1919) ; The Work of a Research Station. *Proc. 4th Intern. Cong. Refrig.* (*London*), **1**, 185—92 (1924) ; A Microscopic Study of the Freezing of Gel. *Proc. Roy. Soc.*, **A 112**, 47—61 (1926).

Hepburn, J. R. I. The Freezing of Inorganic Hydrogels. *Rec. trav. chim.*, **45**, 321—7 (1926).

Hoagland, R., *et al.* Changes in Fresh Beef during Cold Storage above Freezing. U.S. Dept. Agr., *Bull.*, **433**, 1—100 (1917).

Jenkins, M. K., *et al.* Effects of Cold Storage on Shell Eggs. *Ice and Refrigeration*, **58**, 140—7 (1920).

Jong, D. A. de. The Freezing of Meat by Ottesen's Method : Summary of Results. *Proc. 4th Intern. Cong. Refrig.* (*London*), **2**, 1092—4 (1924).

Jorgensen, I., and Stiles, W. Some Scientific Aspects of Cold Storage. *Science Progress*, **13**, 614—20 (1919) ; **14**, 98—105 (1919) ; **14**, 427—34 (1919).

Joslyn, M. A., and Marsh, G. L. Heat Transfer in Foods during Freezing and Subsequent Thawing : I. Temperature Changes in Sugar Solutions, Sweetened Fruit Juices and Other Liquids. *Ind. Eng. Chem.*, **22**, 1192—7 (1930).

Kidd, F. Biology and Refrigeration : Cantor Lectures. *J. Roy. Soc. Arts.*, **77**, 269, 288, 313 (1929).

Kinoshita, K. The Freezing of Gelatin. *Bull. Chem. Soc. Japan*, **5**, 261—6 (1930).

Kremann, R., and Eitel, H. The Ternary System Sugar-Citric Acid-Water. A Contribution to the Theory of Fruit Ices from the Standpoint of the Phase Rule. *Rec. trav. chim.*, **42**, 539—46 (1923).

Kruyt, H. R., and Winkler, K. C. The Influence of Hydrated Colloids on the Freezing-Point Lowering. *Z. anorg. allgem. Chem.*, **188**, 200—4 (1930).

Martel. Frozen Meat, *J. pharm. chim.*, **20**, 62—8 (1919).

Martin, J. W. The Quick Freezing of Perishable Foodstuffs. *Refrig. Eng.* **19**, 131—3 (1920).

Molisch, H. "Untersuchungen uber das Erfrieren der Pflanzen." Jena, 1897.

Moran, T. The Freezing of Eggs. *Proc. 4th Intern. Cong. Refrig.* (*London*), **1**, 122—35 (1924); The Effect of Low Temperature on Hen's Eggs. *Proc. Roy. Soc.*, **B 98**, 436—56 (1925); Science and the Future of the Frozen Meat Industry. *Proc. Brit. Cold Storage and Ice Assoc.*, **1926**, 89—104; The Science of Meat Freezing. *Refrig. Eng.*, **12**, 343—4 (1926); The Freezing of a Gelatin Gel. *Proc. Roy. Soc.*, **A112**, 30—46 (1926); Critical Temperature of Freezing: Living Muscle. *Ibid.*, **B 105**, 177—97 (1929); Recent Advances in the Low-Temperature Preservation of Foodstuffs. *J. Soc. Chem. Ind.*, **48**, 245—51 T (1929); The Frozen State in Mammalian Muscle. *Proc. Roy. Soc.*, **B 107**, 182—7 (1930).

Moran, T., and Smith, E. C. Post-mortem Changes in Animal Tissues: The Conditioning or Ripening of Beef. Dept. Sci. Ind. Res., Food Investgn., *Special Rept.*, No. **36**, 1—64 (1929).

Nuttall, G. H. F., and Gardiner, J. S. The Histological Changes in Frozen Fish and the Alterations in Taste and Physiological Properties of Their Flesh. A *Résumé* of Dr. K. Reuter's Investigations. *J. Hyg.*, **17**, 56—62 (1918).

Parentz, H. The Freezing of Wine, Milk and Other Liquid Foods. *Compt. rend.*, **158**, 921 (1914).

Parfitt, E. H. The Hydrogen Ion Concentration of Cold-Storage Butter. *World's Butter Rev.*, **1**, (3), 11—15 (1927).

Payne, N. M. Some Effects of Low Temperature on Internal Structure and Function in Animals. *Ecology*, **11**, 500—4 (1930).

Pecori, G. Value of Rational Thawing of Frozen Meats for Food. *Ann. Igiene*, **36**, 39—52 (1926).

Pennington, M. E., Hepburn, J. S., St. John, E. Q., and Witmer, E. The Influence of Temperatures Above Freezing on the Changes in Chemical Composition, Bacterial Content, and Histological Structure of the Flesh of the Common Fowl. *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **29**, xxxi—xxxii. (1917). The Influence of Temperatures Below Freezing on the Changes in the Chemical Composition, Bacterial Content, and Histological Structure of the Common Fowl. *Ibid.*, **29**, xxxiii.—xxxiv. (1917).

Perlzweig, W. A., and Gies, W. J. A Further Study of the Chemical Composition and Nutritive Value of Fish Subjected to Prolonged Periods of Cold Storage. *Columbia Univ. Biochem. Bull.*, **3**, 69—71 (1914).

Petersen, P. W. Modern Fish Freezing. *Proc. 4th Intern. Cong. Refrig.* (*London*), **2**, 1181—98 (1924).

Pique, J. The Freezing of Fish on Land and at Sea. *Ibid.*, **2**, 1040—60 (1924).

Plank, R., Ehrenbaum, E., and Reuter, K. "Die Konservierung von Fischen durch das Gefrierverfahren." (Berlin: Zentral-Einkaufsgesellschaft, 1916).

Pupilli, G. The Surface Tension of Protein Solutions at Various Hydrogen Ion Concentrations Before and After Freezing. *Arch. sci. biol.* (*Napoli*), **12**, 373—94 (1928).

Quagliariello, G. Action of Cold on the Fats of Milk. *Atti. accad. Lincei.*, (6) **7**, 75—8 (1928).

Reid., W. H. E. The Deleterious Effect of Freezing on Several of the Physical Properties of Milk. Univ. Missouri Agr. Expt. Sta., *Res. Bull.*, **100**, 1—14 (1927).

Reiner, L. The Behaviour of Pro tein Fractions and Their Immune Reactions on Freezing of the Serum. *Kolloid-Z.*, **32**, 284—6 (1923).

Richardson, W. D., and Scherubel, E. The Deterioration and Commercial Preservation of Flesh Foods. (1) General Introduction and Experiments on Frozen Beef. *J. Am. Chem. Soc.*, **30**, 1515—64 (1908); (2) The Storage of Beef at Temperatures Above the Freezing Point. *Ind. Eng. Chem.*, **1**, 95—9 (1909).

Sacharov, N. L. Studies in Cold Resistance of Insects. *Ecology*, **11**, 505—17 (1930).

Sellschop, J. P. F., and Salmon, S. C. The Influence of Chilling, above the Freezing Point, on Certain Crop Plants. *J. Agr. Research*, **37**, 315—8 (1928).

Sherrill, L. H. Quick Freezing of Filleted Fish. *Food Inds.*, **1**, 33—5 (1928).

Smith, C. S. A Study of the Influence of Cold Storage Temperatures upon the Chemical Composition and Nutritive Value of Fish. *Columbia Univ. Biochem. Bull.*, **3**, 54—68 (1914).

Smith, E. C. The Formation of Lactic Acid in the Muscles in the Frozen State. *Proc. Roy. Soc.*, **B 105**, 198—207 (1929); The Heat of Rigor of Mammalian Muscle. *Ibid.*, **B 107**, 214—22 (1930); On the Coagulation of Muscle Plasma. *Ibid.*, **B 105**, 579—99 (1930).

Sommer, H. H. The Freezing Point of Cheddar Cheese : Injury of Cheese by Freezing. *J. Dairy Sci.*, **11**, 9—17 (1928).

Stiles, W. The Preservation of Food by Freezing with Special Reference to Fish and Meat : A Study in General Physiology. Dept. Sci. Ind. Res., Food Investign., *Special Rept.*, No. **7**, 1—186 (1922).

Taylor, H. F. Solving the Problems of Rapid Freezing. *Food Inds.*, **2**, 146—151 (1930).

Urbain, O. M., and Miller, J. N. Relative Merits of Sucrose, Dextrose and Levulose as Used in the Preservation of Eggs by Freezing. *Ind. Eng. Chem.*, **22**, 355—6 (1930).

Verriere, P. Experiments in the Freezing of Fish at the Lorient Cold Stores. *Proc. 4th Intern. Cong. Refrig.* (*London*), **2**, 1060—72 (1924).

Vickery, J. R. Refrigeration of Meat. II. Freezing of Beef and Mutton Press Juices. *Australian J. Exptl. Biol. Med. Sci.*, **3**, 81—7 (1926).

Vondrak, J. Changes in the Composition of Frozen Beets. *Intern. Sugar J.*, **28**, 149—51 (1926).

Webber, H. J., *et al.* A Study of the Effect of Freezes on Citrus in California. (1) A Study of the Freeze of 1913 in California. (2) Changes that Take Place in Frozen Oranges and Lemons. Calif. Agr. Expt. Sta., *Bull.*, **304**, 245—321 (1919).

Whitcomb, W. O., and Sharp, P. F. Wheat and Flour Studies. VII. Milling and Baking Tests of Frozen and Non-Frozen Wheat Harvested at Various Stages of Maturity. *Cereal Chem.*, **3**, 301—15 (1926).

Wright, A. M. Characteristics and Relative Values of Frozen and Chilled Meats. *Proc. 4th Intern. Cong. Refrig.* (*London*), **1**. 332—4 (1924).

Wright, R. C., and Taylor, G. F. Freezing Injury to Potatoes when Undercooled. U.S. Dept. Agr., *Bull.* **916**, 1—15 (1921).

Wright, R. C. Freezing Injury to Potatoes in Storage or Transit. Am. Assoc. Ice Refrig., *Proc. Ann. Mtg.*, (1924/25) ; **14**, 119—23 (1925) ; Some Effects of Freezing on Onions. U.S. Dept. Agr., *Dept. Circ.*, No. **415**, 1—8 (1927).

Young, H. D. Composition of Frozen Oranges and Lemons. *Ind. Eng. Chem.*, **7**, 1038—41 (1915).

Zarotschenzeff, M. T. " Between Two Oceans : Rapid Chilling and Freezing Systems for Fish and Meat." (London : 1930).

THE PROBLEM OF BOUND WATER

Balcar, J. O., Sansum, W. D., and Woodyatt, T. T. Fever and the Water Reserve of the Body. *Arch. Internal Med.*, **24**, 116—28 (1919).

Gortner, R. A. The Application of Colloid Chemistry to Some Agricultural Problems. *Am. Coll. Symp. Monog.*, **1**, 392—412 (1923) ; The State of Water in Colloidal and Living Systems. *Trans. Faraday Soc.*, **26**, 678—86 (1930).

Hardy, W. B. Free and Bound Liquid in Gels. *Kolloid-Z.*, **46**, 268—77 (1928).

Jackson, C. M. Storage of Water in Various Parts of the Earthworm at Different Stages of Exsiccation. *Proc. Soc. Exptl. Biol. Med.*, **23**, 500—4 (1925).

Marinesco, N. Properties of Dielectrics and the Structure of Hydrophylic Colloids. *Compt. rend.*, **187**, 718—20 (1928) ; Dielectric Polarization and the Structure of Hydrophylic Colloids. *Ibid.*, **189**, 1274—6 (1929).

Newton, R., and Gortner, R. A. A Method for Estimating the Hydrophylic Colloid Content of Expressed Plant Tissue Fluids. *Botan. Gaz.*, **74**, 442—6 (1922).

Newton, R. A Comparative Study of Winter Wheat Varieties with Special Reference to Winter Killing. *J. Agr. Sci.*, **12**, 1—19 (1922) ; Colloidal Properties of Winter Wheat Plants in Relation to Frost Resistance. *Ibid.*, **14**, 178—91 (1924) ; The Nature and Practical Measurement of Frost Resistance in Winter Wheat. Univ. Alberta, Coll. Agr., *Res. Bull.*, **1**, 1—53 (1923).

Robinson, W. Water Binding Capacity of Colloids, a Definite Factor in Winter Hardiness of Insects. *J. Econ. Entomol.*, **20**, 80—8 (1925) ; Water Conservation in Insects. *Ibid.*, **21**, 897—902 (1928) ; Relation of Hydrophylic Colloids to Winter Hardiness of Insects. *Am. Coll. Symp. Monog.*, **5**, 199—218 (1928) ; Response

and Adaptation of Insects to External Stimuli. *Ann. Entomol. Soc. (America)*, **21**, 407—17 (1928).

Thoenes, F. Investigations on the Question of the Binding of Water in Colloids and Animal Tissues. *Biochem. Z.*, **157**, 174—86 (1925); The Influence of Dehydration on the Distribution of Water in the Tissues and on the Production of Intoxication in Young Dogs. *Monatsschr. Kinderheilk*, **41**, 440—9 (1928).

WATER PURIFICATION

Barnitt, J. B., and Haux, E. H. Coagulation with Sodium Aluminate. *Can. Eng.*, **51**, 725—6 (1926).

Brownstead, J. P., *et al.* Use of Sodium Aluminate as Coagulant. Ohio Cong. Water Purifcn., *4th Ann. Rept.* (**1925**), 31—6.

Burdick, C. H. Experiences in Application of Alum in Coagulation. *Water Works Eng.*, **82**, 1629—30, 1633 (1929).

Christman, C. H. Ultramicroscope Studies of Colloids in Water. *J. Am. Water Works Assocn.*, **21**, 1076—80 (1929).

Ellms, J. W. Coagulation and Sedimentation with Chemicals. *J. Am. Water Works Assocn.*, **9**, 496—501 (1922).

Ellms, J. W., and Marshall, L. A. Studies on the Effect of Hydrogen Ion Adjustment on the Coagulation, Sedimentation and Filtration of Lake Erie Water Using Aluminium Sulphate as Coagulant. Ohio Conf. Water Purifcn., *2nd Ann. Rept.* (**1922**), 114—29.

Fleming, J. D. Purification of Highly Turbid Water. *J. Am. Water Works Assocn.*, **22**, 1559—76 (1930).

Hansen, P. Developments in Water Purification Practice. *Eng. News-Record*, **104**, 839—43 (1930).

Hopkins, E. S. Composition of Alum Floc in Mixing Basin. *J. Am. Water Works Assocn.*, **12**, 288—94 (1924); Composition and Use of Ferric Hydroxide as a Coagulant. *Ind. Eng. Chem.*, **21**, 58—60 (1929).

Hopkins, E. S., and Whitmore, E. R. Study of the Floc produced by Chlorinated Copperas. *Ibid.*, **22**, 79—81 (1930).

Moberg, A. R., and Partridge, E. M. Ferric Alumina : a Modern Development in the Field of Coagulation. *Ind. Eng. Chem.*, **22**, 163—4 (1930).

Morse, R. B., Hechmer, C. A., and Powell, S. T. Coagulation Studies at the Washington Suburban Sanitary District. *Ind. Eng. Chem.*, **20**, 56—9 (1928).

Peterson, B. H., and Bartow, E. Effect of Salts on the Rate of Coagulation and the Optimum Precipitation of Alum Floc. *Ind. Eng. Chem.*, **20**, 51—5 (1928).

Powell, S. T. Sodium Aluminate as an Adjunct to Alum for the Coagulation of Public Water Supplies. *Am. J. Pub. Health*, **17**, 804—9 (1927).

Rakusin, M. A. The Adsorption of Proteins, Enzymes, Toxins and Sera by Aluminium Hydroxide. *Z. Immunität.*, **34**, 155—93 (1922).

Ripple, O. J., Turre, G. J., and Christman, C. H. Use of Liquid Sodium Aluminate in the Clarification of the Denver Water Supply. *Ind. Eng. Chem.*, **20**, 748—52 (1928).

Schade, H., and Lohfert, H. Ultraviolet Tyndall Cone of Pure Water. *Kolloid-Z.*, **51**, 65—71 (1930).

Sierp, F. Water and Sewage. Liesegang's "Kolloidchemische Technologie" (Dresden, 1931).

Stumper, R. Barium Aluminate and its Use in Water Purification. *Chimie et industrie*, **22**, 1067—83 (1929).

Vasiliev, P. I. Sodium Aluminate for the Purification of Water for Drinking. *Zhur. Prikladnoi Khimii*, **3**, 207—19 (1930).

Wolman, A., Donaldson, W., and Enslow, L. H. Recent Progress in the Art of Water Treatment. *J. Am. Water Works Assocn.*, **22**, 1161—77 (1930).

ALUMINIUM HYDROXIDE

Archibald, E. H., and Habasian, Y. Studies on the Solubility of Aluminium Hydroxide. *Trans. Roy. Soc. Canada*, **11**, 1—6 (1917).

Böhm, J., and Niclassen, H. Amorphous Precipitates and Crystallised Sols. *Z. anorg. allgem. Chem.*, **132**, 1—9 (1923).

Britton, H. T. S. The Precipitation of the Hydroxides of Zinc, Chromium, Beryllium, Aluminium, Bivalent Tin and Zirconium by the Use of the Hydrogen Electrode and Their Alleged Amphoteric Nature. *J. Chem. Soc.*, **127**, 2120—41 (1925).

Buswell, A. M., and Edwards, G. P. Some Facts about Residual Alum in Filtered Water. *Chem. Met. Eng.*, **26**, 826—9 (1922).

Euler, H. V., and Nilsson, R. Equilibrium of Colloidal Aluminium and Lanthanum Hydroxides with Dilute Acids and Bases. *Ber.*, **57**, 217—22 (1924).

Fricke, R., and Wever, F. X-ray Spectrographic Investigations of the Effect of Ageing on Precipitated Metal Hydroxides. *Z. anorg. allgem. Chem.*, **136**, 321—4 (1924).

Grobert, E. Reactions of Caustic Soda with the Aluminium Salts. *J. chim. phys.*, **19**, 331—5 (1922).

Hatfield, W. D. Soluble Aluminium and the Hematoxylin Test in Filtered Waters. *Ind. Eng. Chem.*, **16**, 233—4 (1924).

Heyrovsky, J. The Acidity and Constitution of Aluminic Acid. *J. Chem. Soc.*, **117**, 1013—25 (1920).

Howard, N. J., and Haman, F. Alumina Hydrate in Mechanically Filtered Water. *Can. Eng.*, **38**, 461—2 (1920).

Johnston, E. W., and Downey, F. P. Use of Aluminium Hydroxide Sol. *Can. Eng.*, **50**, 94C—94D (1926).

Kohlschutter, V. Disperse Aluminium Hydroxide. *Z. anorg. allgem. Chem.*, **105**, 1—25 (1918).

Kraut, H. The Chemistry of Alumina Hydrogels. *Centr. Mineral Geol.*, **A (1926)**, 64—80.

Laporte, A. V. de, and Manuel, F. R. Colloid Chemistry in Filtration. *Can. Eng.*, **50**, 94A—94C (1926).

Massink, A. The Minimum Solubility of Aluminium Hydroxide in Water. *Chem. Weekblad.*, **19**, 66 (1922).

Miller, L. B. Adsorption by Aluminium Hydroxide Considered as a Solid Solution Phenomenon. *U.S. Pub. Health Repts.*, **39**, 1502—16 (1924); A Study of the Effects of Anions Upon the Proper-

ties of Alum Floc., *Ibid.*, **40,** 351—67 (1925); The Effect of Anions upon the Physical, Chemical and Colloidal Properties of Aluminium Hydroxide. *Am. Coll. Symp. Monog.*, **3,** 208—15 (1925).

Moser, H. Studies in Buffer Action: Aluminium Hydroxide. *Helvetica Chim. Acta.*, **10,** 310—19 (1927).

Prakash, S., and Dhar, N. R. Changes in the Viscosity and Hydrogen Ion Concentration of Some Inorganic Substances During the Process of Jelly Formation. *J. Indian Chem. Soc.*, **6,** 391—409 (1929).

Sen, K. C. The Solubility of Colloidal Solutions: Aluminium Hydroxide Suspensions. *J. Phys. Chem.*, **28,** 1029—35 (1924).

Shidei, J. The Composition of a Precipitated Aluminium Hydroxide. *Mem. Coll. Sci. Kyoto Imp. Univ.*, **9A,** 42—73 (1924).

Slade, R. E., and Polack, W. G. The Acidic and Colloidal Properties of Aluminium Hydroxide. *Trans. Faraday Soc.*, **10,** 150—4 (1914).

Spencer, H. M. Properties and Uses of Colloidal Aluminium Hydroxide. *Chem. Age (N.Y.)*, **32,** 31—3 (1924).

Theriault, E. J., and Clark, W. M. An Experimental Study of the Relation of Hydrogen Ion Concentrations to the Formation of Floc in Alum Solutions. *U.S. Pub. Health Repts.*, **38** (5), 181—98 (1923).

Werner, H. Liquid Films and Clarification. *Kolloid-Z.*, **50,** 247—62 (1930).

Williamson, F. S. Basic Aluminium Sulphate. *J. Phys. Chem.*, **27,** 284—9 (1923).

Wolman, A., and Hannan, F. Residual Aluminium Compounds in Water Filter Effluents. *Chem. Met. Eng.*, **24,** 729—35 (1921).

INFLUENCE OF pH

Bachmann, E. E. J. The Influence of pH on the Clarification of Water. *Rev. facultad. cienc. quim.*, **3,** 107—15 (1925).

Bakhuygen, W. H. V. S. The Hydrogen Ion Concentration of the Aqueous Solution of an Amphoteric Oxide. *Chem. Weekblad.*, **19,** 41—3 (1922).

Banerji, L. N. The Influence of Hydrogen Ion Concentration on the Dose of Alum and the Mechanism of the Action of Alum in the Clarification of Natural Waters. *Indian J. Med. Research,* **11,** 695—718 (1924).

Cady, H. P., Kemmerer, G., and Weeks, M. E. The *Rôle* of Hydrogen Ion in the Precipitation of Calcium and Magnesium Carbonates. *J. Phys. Chem.*, **33,** 1769—80 (1929).

Carpenter, L. V. The Use of pH Control in a Sanitary Laboratory. W. Va. Coll. Eng., *Tech. Bull.*, **1,** 55—65 (1927).

Catlett, G. F. Coagulation Control by Hydrogen Ion Concentration. *Eng. Control,* **60,** 281—5 (1923); Optimum Hydrogen Ion Concentrations for the Coagulation of Various Waters. *J. Am. Water Works Assocn.*, **11,** 887—95 (1924).

Ellms, J. W., and Marshall, L. A. Studies on the Effect of Hydrogen Ion Concentration Adjustments on the Coagulation, Sedimenta-

tion and Filtration of Lake Erie Water, Using Aluminium Sulphate as the Coagulant. Ohio Conf. Water Purifcn., *2nd Ann. Rept.*, (1922), 114—29.

Greenfield, R. E., and Buswell, A. M. Investigation by Means of the Hydrogen Electrode of the Chemical Reactions Involved in Water Purification. *J. Am. Chem. Soc.*, **44**, 1435—42 (1922).

Hatfield, W. D. Relation of Hydrogen Ion Concentration to Filter Plant Operation. *Ind. Eng. Chem.*, **14**, 1038—40 (1922).

Mortensen, F. C. Hydrogen Ion Concentration and Water Purification at Cedar Rapids, Iowa. *Am. Water Works Assocn.*, **13**, 33—8 (1925).

Saunders, J. T. The Hydrogen Ion Concentration of Natural Waters : The Relation of pH to the Pressure of Carbon Dioxide. *Brit. J. Exptl. Biol.*, **4**, 46—72 (1926).

Wagner, A., and Enslow, L. H. Applied Hydrogen Ion Control : Study of its Merits in Practical Filter Plant Operation. *J. Am. Water Works Assocn.*, **9**, 373—91 (1922).

ELECTROÖSMOSIS

Aten, A. H. W. Electroösmotic Water Purification. *Chem. Weekblad.*, **25**, 211—9 (1928).

Bartow, E. Electroösmose Treatment of Boiler Waters. *J. Am. Water Works Assocn.*, **22**, 115—21 (1930).

Bartow, E., and Jebens, R. H. Purification of Water by Electro-ösmosis. *Ind. Eng. Chem.*, **22**, 1020—2 (1930).

Behrman, A. S. The Purification of Water by Electroösmosis. *Chem. Age*, **21**, 305—7 (1929) ; *Ind. Eng. Chem.*, **19**, 1229—30 (1927) ; *J. Chem. Education*, **6**, 1611—8 (1929).

Bezold, V. Electroösmotic Purification of Water. *Brennstoff-Wärmewirt*, **8**, 242—5 (1926).

Burkes, J. Electroösmotic Water-Purifying Plates. *Chem. App.*, **17**, 85—7 (1930).

Chemnitius, F. Electrophoresis. *Chem.-Ztg.*, **53**, 361—2, 378—80 (1929).

Illig. K. Electroösmotic Processes on a Commercial Scale. *Z. angew. Chem.*, **39**, 1085—93 (1926) ; The Electroösmotic Purification of Water by the Siemens-Elektro-Osmose G.m.b.H. Process. *Siemens-Z.*, **8**, 349—58 (1928) ; The Preparation of Distilled Water by Electro-Endosmosis. *Chem. Listy*, **23**, 408—16 (1929).

Mayer, E., and Schön, R. A New Method for the Purification of Water. *Oesterr. Chem.-Ztg.*, **27**, 46—7 (1924).

Patin, P. Preparation of Pure Water Without Distillation : Electroösmosis. *Chimie et industrie*, **19**, 205—13 (1928).

Remy, H. Hydration Problem : (1) Calculation of the Quantity of Water Involved by Ions from Electrical Mobilities. *Z. physik. Chem.*, **89**, 467—88 (1915) ; (2) A New Method for the Determination of the Electrolytic Transport of Water. *Ibid.*, **89**, 529—69 (1915) ; Electro-Endosmosis and the Electrolytic Transfer of Water. *Z. Elektrochem.*, **29**, 364—70 (1923) ; *Z. physik. Chem.*, **118**, 161—92 (1926).

Riediger, O. Purification of Water by Electroösmosis. *Naturforscher.*, **1929**, 314—6.

USE OF ACTIVE CARBON

Bailleul, G. The Use of Active Carbon for the Purification of Water. *Water Water Eng.*, **32**, 424—6 (1930).

Bayliss, J. R. Further Observations on the Use of Activated Carbon in Removing Objectionable Tastes and Odours from Water. *J. Am. Water Works Assocn.*, **22**, 1438—61 (1930).

Behrman, A. S., and Crane, H. B. Chemical and Mechanical Utilisation of Activated Carbon in Water Purification. *J. Am. Water Works Assocn.*, **22**, 1399—1413 (1930).

Carpenter, L. V. Use of Activated Carbon in Water Treatment. W. Va. Eng. Expt. Sta., *Tech. Bull.*, **3**, 25—34 (1930).

Hansen, P., *et al.* Discussions of Papers on Activated Carbon. *J. Am. Water Works Assocn.*, **22**, 1462—75 (1930).

Jotten, K. W., *et al.* The Removal of Polluting Substances in Drinking Water by Active and Non-Active Carbon. *Gesundh. Ing.*, **52**, 529—34, 546—50 (1929); **53**, 227—34 (1930).

Koenig, O. Improvements in Chemical Water Purification and the Use of Active Carbons and Earths. *Gas Wasserfach*, **72**, 1065—72, 1091—1102 (1929).

Narcom, G. D., and Dodd, R. I. Activated Carbon for the Removal of Taste and Odour. *J. Am. Water Works Assocn.*, **22**, 414—37 (1930).

FACTORY EFFLUENTS

Bartow, E. Disposal of Waste from the Sugar-Beet Industry. *Chem. Age (N.Y.)*, **32**, 373—5 (1924); Disposal of Some Organic Trade Wastes. *Ind. Eng. Chem.*, **18**, 1085—6 (1926).

Bode, G. Purification of Waste Waters from the Fermentation Industries. *Z. angew. Chem.*, **40**, 84—7 (1927).

Burroughs, L. C. The Disposal of Dairy Wastes. *J. Dairy Sci.*, **7**, 503—23 (1924).

Buswell, A. M., Greenfield, R. E., and Shive, R. A. Chemical Characteristics of Some Trade Wastes. *Ind. Eng. Chem.*, **18**, 1082—4 (1926).

Eldridge, E. F. Studies on the Treatment of Milk-Product Waste. Michigan State Coll. Eng. Expt. Sta., *Bull.*, **24**, 3—26 (1929); *Bull.*, **28**, 3—31 (1930).

Ferguson, R. H. Lime in the Treatment of Trade Wastes. *Rock Products*, **17** (13), 43—5 (1924).

Hatfield, W. D. Some Observations on Beer-Slop Wastes from Corn Mash Distillation. *Ind. Eng. Chem.*, **22**, 276—7 (1930).

Hedley, E. P. Treatment of Mill Effluents. *S. Afr. Sugar J.*, **14**, 317, 319, 321, 323 (1930).

Hommon, H. B. Purification of Creamery Wastes. U.S. Pub. Health Service, *Pub. Health Bull.*, **109**, 1—87 (1921).

Jaskólski, J. The Treatment of Waste Waters from Sugar Refineries. *Listy Cukrovar*, **48**, 1—7, 9—14 (1929).

Kimberley, A. E. Methods in Milk Waste Treatment. *Munic. Sanitation*, **1**, 89—92 (1930); Progress Report on Cannery Waste Treatment Studies. State of Ohio, Dept., Health, Columbus, Ohio (**1927**), 1—85.

Manuelli, C., and Anselmi, S. Waste Water from Sugar Factories. *Att III. congresso naz. chim. pura. applicata* (**1930**), 607—26.

Mohlman, F. W. Treatment of Packing-House, Tannery and Corn Products Wastes, *Ind. Eng. Chem.*, **18**, 1076—81 (1926).

Slaughter, C. E. Disposal of Creamery Wastes. Michigan State Coll. Eng. Expt. Sta., *Bull.*, **18**, 1—27 (1928).

Steel, E. W., and Zeller, P. J. A. The Treatment of Dairy Waste. Texas Eng. Expt. Sta., *Bull.*, **38**; Series **4**, Vol. I., No. 5, 1—16 (1930).

Topinka, F. The Waste Waters from Chemical Industries. *Chem. Listy.*, **23**, 451—61 (1929).

Underwood, A. J. V. The Disposal of Effluents from Sugar-Beet Factories. *Ind. Chem.*, **3**, 260—7 (1927).

Walker, C. L. Treatment of Dairy Wastes. N.Y. State Sewage Works Assocn. *Reprint* (**1929**), 1—13.

Warrick, L. F. Lime in the Treatment of Pea Cannery Wastes. *Ind. Eng. Chem.*, **19**, 577—83 (1927)

Webster, R. Tomato Cannery Waste Treatment Plant. *Proc. 3rd Ann. Conf. Maryland Water Sewerage Assocn.*, **1929**, 33—9.

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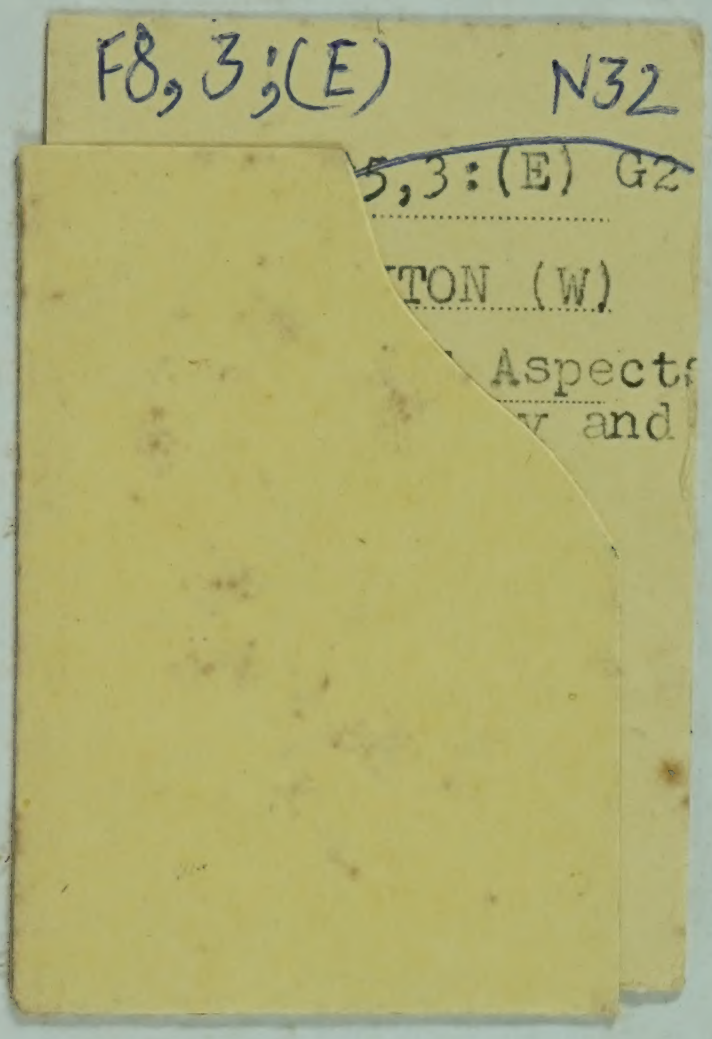
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